RESTRICTED FEEDING, SPERMATOGENESIS AND GROWTH IN ARCTIC CHARR, Salvelinus alpinus: THE IDENTIFICATION OF TWO POSSIBLE GAMETOGENIC CONTROL POINTS

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RESTRICTED FEEDING, SPERMATOGENESIS AND GROWTH IN ARCTIC CHARR, *Salvelinus alpinus*: THE IDENTIFICATION OF TWO POSSIBLE GAMETOGENIC CONTROL POINTS

By

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the Degree of Master of Science.

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ABSTRACT

Experiments were conducted to determine if food restriction during certain critical critical seasons could reduce the incidence of early maturity in Arctic charr (*Salvelinus alpinus* L.) and to describe the stages of spermatogenesis associated with those critical periods.

A seven-stage description of the spermatogenic process is presented along with a time series of the progression through the various stages. Spermatogenesis in Arctic charr is initiated in autumn under decreasing photoperiods and temperatures, approximately one year before spawning. The onset is characterized by mitotic proliferation of spermatogonia before changes in GSI are detectable. The spermatogenic cycle is highly asynchronous within the testis of a single fish, and between individuals of a population. Two critical points in the spermatogenic cycle that may be susceptible to nutritional control were identified. The first occurs in autumn, affecting the advancement of testes from the pre-spermatogonial stage to the immature stages; the second in spring, when spermatogonial cysts form and a switch from spermatogonial proliferation to spermatocyte formation ensues. Reduced feeding temporarily interrupts mitotic division of germ cells during the early stages of gonadal development.

The Arctic charr, aged 1+ in autumn, were subjected to two different restricted feeding regimes. Experiment I, consisting of alternating two week periods of food deprivation with excess feeding (2:2, 14 week duration), at any time from November to June

did not reduce the proportions of male fish showing definite signs of maturity by July compared to a control group. In Experiment II, fish subjected to 6-week periods of continuous starvation (6:0) from September to April exhibited temporary reductions in gonadal activity. By July, final maturation proportions of starved fish were depressed relative to the control group. Restricted feeding had no effect on the degree of gonadal investment in terms of gonadosomatic index (GSI). No females matured during either experiment.

Temporal changes in fish growth were monitored over the course of both studies by retrospective examination of tagged individuals. After periods of food deprivation, resumption of regular feeding resulted in fish displaying hyperphagic feeding activity and compensatory growth. Growth differentials were most pronounced in fish starved during periods of higher temperature, especially during the time of rapid temperature increase in May/June. In Experiment I, maturing males continued to grow and increase body condition throughout the winter months, while growth of their immature counterparts remained low until spring. Growth rates of maturing fish were consistently higher than immature fish. In Experiment II, growth patterns of maturing and immature males were similar, however, condition factors of maturing males were slightly above those of immature fish.

This study lends support to the theory that there are two critical periods in the maturation cycle of salmonids. Food restriction alone, however, is not an effective method of suppressing early maturity in this species owing to the long and flexible duration of the identified critical decision periods.

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CF	condition factor
GSI	gonadosomatic index
L	Leydig cells
Lo	lobular boundaries / wall
Lu	lobular lumen
Mi	mitotically dividing cell
MiG	group mitoses
ScA	primary spermatocytes
ScB	secondary spermatocytes
SgA	primary spermatogonium(ia)
SgB	secondary spermatogonium(ia)
SGR	specific growth rate
St	spermatids
Sz	spermatozoa

1.0 INTRODUCTION

1.1 NUTRITION AND REPRODUCTION

Reproductive processes are energetically expensive and rely heavily on both energy reserves and successful attainment of exogenous resources (Shul'man 1974). The association between nutrition and reproduction has led to extensive investigation into the links between these two systems, especially in mammals (reviewed by I'Anson *et al.* 1991). Possible links identified range from interactions between body state and reproduction, for example, weight and proximate composition (Frisch and Revelle 1970), to the influence of gut peptides acting on the central nervous system (Kennedy and Mitra 1963). The most thoroughly investigated links are those associated with the roles of adiposity and growth. The initiation of puberty in females has been correlated with the attainment of a critical body weight in humans, sheep, rats, chipmunks, and in sheep to the attainment of a minimum lean to fat ratio and percent body fat (I'Anson *et al.* 1991). In sows, nutritional strategies aimed at increasing body fat reserves have been shown to improve fertility (Odowd 1997). Nutritional influences on reproduction have also been identified in amphibians and reptiles (Whittier and Crews 1987). In the alpine smooth snake (*Coronella austriaca*), reproduction is associated with energy levels and condition. Snakes that recovered condition sooner in the year after reproduction were able to reproduce again after a briefer delay (Luiselli 1996).

The role of nutrition on reproduction in teleosts has been studied less extensively, but accumulating evidence suggests a close relationship.

Rejecting opportunities for reproduction is an occurrence which has been reported for a wide range of iteroparous animals (Bull and Shine 1979). In teleosts, inadequate nutrition has been implicated as a causative factor for such omissions. Fedorov (1971) reported that sexually mature, female Greenland halibut (*Reinhardtius hippoglossoides*) of the Barents Sea did not spawn annually. A similar situation has been described for other wild populations of marine fish including the orange roughy, *Hoplostethus atlanticus* (Bell *et al.* 1992), and in Newfoundland populations of winter flounder, *Pleuronectes americanus* (Burton and Idler 1984). Failure of post-mature females to develop vitellogenic oocytes and spawn annually has been investigated experimentally for haddock, *Melanogrammus aeglefinus* (Hislop *et al.* 1978; Hislop 1988), winter flounder (Burton and Idler 1987), and plaice, *Pleuronectes platessa* (Horwood *et al.* 1989). In each case, these conditions were induced by reducing food supplies, providing evidence of a connection between nutrition and reproduction.

The occurrence of non-annual spawning has also been reported for freshwater fish, most notably in relation to the tendency of northern iteroparous fish to spawn at intervals of two or more years. This phenomenon has been documented in Arctic charr, *Salvelinus alpinus*, in France (Jamet 1995), in Norway (Jørgensen *et al.* 1997) and in Nauyuk Lake, Canada (Dutil 1986). Dutil (1983, 1986) suggested that gonadal development drains reserves

to such an extent that the charr are energetically prevented from maturing two years in succession. The same has been reported for several species of anadromous whitefish, *Coregonus artedii*, *C. clupeaformis*, and *Prosopium cylindraceum* (Morin *et al.* 1982; Kennedy 1949, 1953) living in northerly, low productivity environments. Sturgeon, *Acipenser fulvescens* and *A. transmontanus*, represent an extreme example of non-annual spawning. In the wild, females of each species only spawn every four to 11 years (Bull and Shine 1979). Even in captivity under ideal feeding conditions, the spawning interval of sturgeon is difficult to reduce below two years (Williot and Brun 1998). It may be that the energy drain of gonad development and spawning is so great that at least two years are required before the fish are re-conditioned enough to reproduce again, similar to what is seen with the northerly fish. Non-annual spawning has also been noted for more southerly iteroparous salmonids, including Atlantic salmon, *Salmo salar*, and *Salvelinus malma* (Bull and Shine 1979).

Nutrition also appears to play a pivotal role in determining fecundity in teleosts. Scott (1962) and Bagenal (1969) found that in rainbow trout (*Oncorhynchus mykiss*), and in brown trout (*Salmo trutta*), respectively, reduced feeding resulted in decreased fecundity. Similarly, reducing rations are reported to have affected egg production and maturation proportions in the three-spined stickleback, *Gasterosteus aculeatus* (Wootton 1973), haddock, (Hislop 1988), winter flounder (Tyler and Dunn 1976), herring, *Clupea harengus* (Ma *et al.* 1998), Atlantic cod, *Gadus morhua* (Kjesbu *et al.* 1991; Karlsen *et al.* 1995),

plaice (Horwood *et al.* 1989), and Atlantic salmon (Bromage *et al.* 1992).

Precocious maturation of male salmonids, especially under hatchery conditions, has led to extensive investigation into the roles of enhanced feeding opportunity and growth on maturation. Rowe and Thorpe (1990a) found that spring growth was important for determining the onset of maturation of male Atlantic salmon parr. Subsequent to this, it was shown that increasing fat stores or the rate of acquisition of fat in spring may be the determining factor (Rowe *et al.* 1991). Condition factors one year prior to spawning (autumn) have also been implicated (Bohlin *et al.*1994).

Empirical studies on a wide range of animals clearly illustrates that nutrition can play a regulatory role on reproductive processes, even from the larval stage of the life cycle. However, most of this information is based on correlations between some aspect of somatic state (e.g., size, fatness, growth rate) and reproduction with little consideration as to how this physical information is transmitted to the reproductive axis. Determining the physiological mechanisms responsible for this interaction has been proposed as a challenge to animal physiologists and represents one of the great frontiers of biology (I'Anson *et al.* 1991).

In recent years, much excitement has been generated by the identification of the protein leptin and its possible role in the regulation of food intake and body mass in mammals and in the understanding of the relationship between reproductive status and the neuroendocrine system. Dietary restriction in rats is observed to be associated with low plasma leptin levels and sexual immaturity. In these animals, central infusion of leptin was

able to induce sexual maturation (Aubert 1998). In mice it is suggested that rising plasma levels of leptin represents a signal to the brain that the animal is metabolically ready for sexual maturity, and the onset of puberty ensues (Gruaz *et al.* 1998). Low levels of circulatory leptin have also been implicated in menstrual dysfunction in women (Tataranni 1997). It has not yet been demonstrated how this protein can act as a signal of metabolic status, but it has been suggested that it may function by affecting neuropeptide-Y neurons in the hypothalamus and/or it may affect peripheral endocrine targets, such as the pituitary, ovary, testes or pancreas (Aubert 1998).

In teleosts, no such metabolic messenger has yet been described, but it is possible that such a somatic-reproductive link is conserved in vertebrates and does exist. However, the reproductive systems of fish are more diverse and less understood than mammals so before such a regulatory factor can be sought, it is important to understand the reproductive cycle of the species in question. Knowing when the reproductive cycle may be sensitive to nutritional status is essential before one can attempt to identify metabolic signals that may be acting at that time.

1.2 Critical Period

Attempts to decipher the link between metabolic state and reproduction in fish has led to the development of the concept of a maturational 'critical period' (Thorpe 1986, 1994; Burton 1994). This refers to the time when fish may be sensitive to nutritional status and

use this information in making the physiological decision of whether or not to mature for a given year. The determination of nutritionally sensitive critical periods in the life cycles of teleosts is a crucial step towards the eventual identification of metabolic signals relaying information about somatic condition to the reproductive system.

In winter flounder, Burton (1994) showed that a non-reproductive fish is likely to occur if feeding is restricted prior to and immediately subsequent to the current spawning season (mid-April). Feeding manipulations during this time have been successful in inducing the non-reproductive state in these fish, a state that can be subsequently reversed the following year with return to adequate nutrition (Burton 1991). Histological observations of the ovary during this critical time indicate that the non-reproductive state occurs as a result of fish failing to undergo exogenous vitellogenesis, indicating that nutritional status acts as a control mechanism early in the gametogenic process. The failure of fish with high post-winter condition to become non-reproductive when starved during this critical period suggests that it is not feeding level, but rather some measure of current nutritional status that is acting as a reproductive regulator (Burton 1994).

Critical periods for the nutritional control of maturation have also been proposed for salmonids. Since salmonid maturation was thought to be initiated under increasing photoperiods (Scott and Sumpter 1983), it has been proposed that a critical period for Atlantic salmon maturation should occur in spring (Thorpe 1986). This theory was developed in response to two observations made on salmonid life histories. The first was that

something other than photoperiod must be controlling the onset of gametogenesis in fish; otherwise, all fish would mature the first time they experienced the appropriate light conditions. The second stemmed from the idea of age or size at maturity. Thorpe (1986) reasoned that size at age is an *a priori* argument and that fish, before initiating maturation, must in some way assess its physiological state. One suggestion was that this assessment is based on the rate of storage, or turnover of surplus energy, exceeding a genetically determined threshold during a critical season, defined by the rate of increase in day length. He defined this time as the 'seasonal window' for initiation of salmon parr maturation.

This model has now been extensively tested on Atlantic salmon (Adams and Thorpe 1989; Rowe and Thorpe 1990a,b; Herbinger and Friars 1992; Simpson 1992; Berglund 1995; Kadri *et al.*1996) and it was found that feeding opportunity in late winter/spring can have an effect on the proportion of fish maturing in a given year. However, the timing and extent of food restriction required to produce this effect, and the proportions of immature fish resulting, have not been consistent between studies so a set nutritionally sensitive 'critical period' remains obscure. Since maturation may be dependent on other factors such as temperature, photoperiod, and genetics, it is important to know which stage of gametogenesis is being affected. In this way, a critical period can be identified for a given population by recognizing when the fish are at a gametogenic stage susceptible to feed deprivation.

The recent realization that the annual cycle of gonadal growth in salmonids begins in autumn rather than under the increasing day-lengths of spring has led to a revision of

Thorpe's (1986) critical period model in that the decision taken at this time is not whether or not to initiate maturation, but if it should be permitted to continue (Thorpe 1994). There are, to date, no published accounts providing evidence that a critical period may also occur in autumn controlling the onset of early gonadal growth, but the possible existence of two primary annual switch points controlling reproductive function in salmonids is postulated (Thorpe *et al.*1998).

Early maturity is an undesirable trait in cultured fish. Such fish show deteriorating flesh quality, lower growth rates, smaller size, aggressive behaviour and impaired smolting, all of which interrupt production schedules for farmers (Aksnes *et al.* 1986). The ability to control maturational problems through nutritional mechanisms could reduce reliance on the need to use other more labour intensive procedures such as triploidization or hormonal manipulations. Therefore, interest in the identification of a critical period in salmonids and the mechanism controlling the onset of maturation remains high.

1.3 SPERMATOGENESIS

The process of spermatogenesis in teleosts is not well understood, owing to the diversity of the group and thus, a wide range of reproductive strategies (Pudney 1995). Several studies of spermatogenesis in fish have been conducted on salmonids (Henderson 1962; Grier 1981; Billard 1992), however, descriptions of the process are still confusing and inconsistent, and controlling mechanisms still undefined (Pudney 1995). Most studies of

spermatogenesis that do exist are concentrated on later stages of the process when hormonal and steroid profiles are apparent and the testes well developed. Very little information is available on the early stages of gonadal development (Chiba *et al.* 1997). Miura *et al.* (1997) proposed that in Japanese eel, *Anguilla japonica*, entry into spermatogenesis is initiated by gonadotropins stimulating Leydig cells to produce 11-ketotestesterone which in turn induces spermatogenesis through the activation of Sertoli cells. However, this model is far from complete. It does not address many of the still remaining questions about control mechanisms within the reproductive system. Confusion in the literature still remains as to the specific events occurring during spermatogenesis. For example, how and when spermatogonial renewal takes place (Henderson 1962; Grier 1981), the timing of gonadal recrudescence (Scott and Sumpter 1983; Thorpe 1994; Thorpe *et al.* 1998) and the factors regulating entry of germ cells into mitosis and meiosis (Miura *et al.* 1997). The identification of a nutritional critical period corresponding with certain stages of the spermatogenic cycle could help decipher where control mechanisms in the cycle act.

1.4 Arctic Charr

Arctic charr are the most northerly distributed freshwater fish with a circumpolar range in the Northern hemisphere (Scott and Crossman 1973). Unless landlocked, most strains are anadromous, migrating downstream after spring thaw and entering the ocean for a summer feeding season. Juvenile fish usually remain in freshwater for a number of years

before making their first seaward migration. In the marine environment, Arctic charr are not highly migratory, remaining close to shore and their native streams to which they return in mid- to late-summer to overwinter (Leim and Scott 1966). Anadromous strains of Arctic charr from the Fraser River, Labrador, average 6.9 years and 38.1 cm at maturity (Delabbio 1995). In Arctic waters, charr spawn in early autumn, usually in September or October; in more southerly regions, spawning extends later into autumn, occurring in November or December (Scott and Crossman 1973). Spawning is reported to occur over gravel or rocky shoals when water temperatures reach 4°C (Scott and Crossman 1973; Jobling *et al.* 1998). The eggs develop over the winter months and fry hatch in early spring (Jobling *et al.* 1998).

Arctic charr are morphologically and physiologically similar to Atlantic salmon and rainbow trout. Since the 1970s, they have been regarded as a promising candidate for aquaculture, owing to higher growth rates in cold water than either salmon or trout (Delabbio 1995). However, the expansion of charr farming has not been as rapid as originally predicted. In a recent review, Jobling *et al.* (1998) outlined the problems with charr culture which have troubled the industry. One of the main problems described is early maturity. The provision of good growth conditions allow charr to reach in one year the size it would normally require several years in the wild to attain, an occurrence which has been associated with high percentages of early maturing male fish. Because of this tendency for early maturation under accelerated growth regimes, and the paucity of information on the gametogenic and reproductive cycles of Arctic charr, it was decided that this species would

make a good model fish on which to conduct feed restriction and gonadal development studies.

1.5 Objectives

The primary purpose of this study was to examine the effect of food restriction on reproductive development in Arctic charr. The specific objectives were:

1. To define the critical period(s) in the gametogenic cycle of charr;
2. To identify the stage(s) of the spermatogenic cycle affected by food restriction;
3. To describe the annual cycle of spermatogenesis in Arctic charr;
4. To examine the effects of restricted feeding on growth and performance.

2.0 Materials and Methods

2.1 Fish and Holding Conditions

Hatchery raised Arctic charr from Daniel's Harbour Arctic Charr Hatchery, Newfoundland, were used in this study. The strain originated from the Fraser River, Labrador (56°39'N, 63°10'W). The fish used in Experiment I were fertilized in the fall of 1994 at Hidden Valley Charr Farms, PEI, and shipped to Daniel's Harbour where they hatched in winter 1995. They were obtained from the hatchery in October 1996 at age 1+. Fish for Experiment II were fertilized in Daniel's Harbour in Fall 1995 and hatched in winter 1996. In September 1997 they were obtained from the hatchery at age 1+. In both years, the average initial weight of the fish was 18g and represented the mid-sized range of fish from the individual cohorts. That is, the largest and smallest fish of each individual year class were not used in this study.

Experiments were conducted at the Ocean Sciences Centre of Memorial University of Newfoundland, Logy Bay, NF (47°30'N, 52°10'W) under a simulated natural photoperiod maintained by a photocell controlled timing system. The fish were held in 1m × 1m (270*l*) fiberglass tanks supplied with flow-through freshwater at rates of approximately 8 $l \cdot min^{-1}$. The water temperature profile for both years is shown in Figure 1. The fish were held under ambient water temperatures uUntil May of each year when a heat exchanger was installed

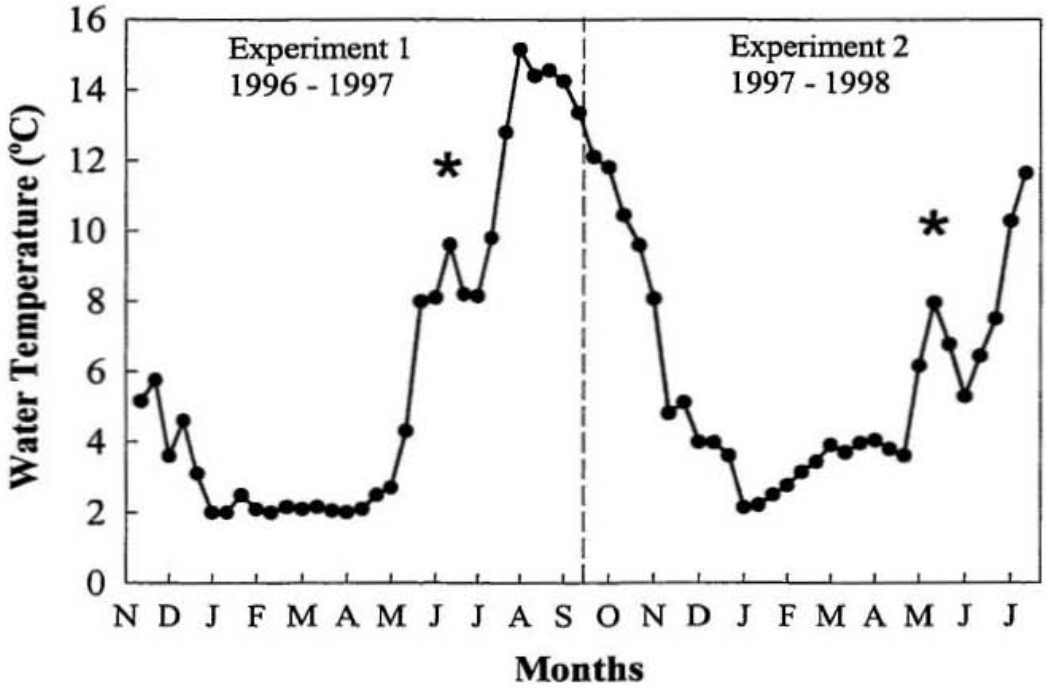


Figure 1: Mean water temperatures for 10-day intervals throughout both experimental periods. Vertical line represents time of division between Experiments I and II; asterisks indicate time of heat exchanger installation.

to cool the water. This system worked effectively until salt-water temperatures increased beyond 10°C in late July. Low dissolved oxygen concentrations (50% saturation) associated with elevated water temperatures (max. 16°C) in late summer of 1997 necessitated the installation of an oxygen injection system, which brought oxygen levels back to 90%.

It was necessary to keep water temperatures below 12°C to prevent the onset of proliferative kidney disease, a disease caused by the parasitic organism PKX, which is endemic to the water supply at the Ocean Sciences Centre (Brown *et al.* 1991). Regardless of efforts made to circumvent the onset of this disease, mortalities occurring in late summer (September 1997) in Experiment I were attributed to this cause.

2.2 Experimental Design

This study consisted of two experiments. Experiment I was conducted in the period of November 4, 1996, to October 2, 1997; Experiment II ran from September 27, 1997, to July 10, 1998.

2.2.1 *Experimental Set-up*

Fish were stocked into 12 tanks (5 treatments plus a control, in duplicate) according to the distribution outlined in Table 1. In Experiment I water depth was adjusted to ensure stocking densities were comparable between tanks (initially ~ 24 kg/m^3). Treatment consisted of restricted feeding of the fish in the five pairs of experimental tanks during

different time periods. Control fish were fed to excess throughout the experiment, with excess feeding defined as feeding to the point where fish left food remaining on the bottom of the tank. Observations indicated that fish were capable of, and did feed from the bottom of the tanks. Extra food was removed from the tanks daily by siphoning. Throughout both experiments fish were fed a commercially prepared trout diet (Corey Vigor Trout Feed, Corey Feed Mills, New Brunswick) containing ~ 40% crude protein and ~18% lipid.

Table 1: Number of fish per group at the beginning of each experiment. Groups were divided into two replicate tanks each.

Experiment	Control Tanks	Treatment tanks				
		Group 1	Group 2	Group 3	Group 4	Group 5
I	624	280	260	240	220	200
II	306	306	306	306	306	306

Twenty fish from each tank were individually tagged using Floy® Fingerling tags stitched through the dorsal musculature immediately anterior to the dorsal fin. For tagging and periodic measurements, fish were anaesthetized using 2-phenoxyethanol at a concentration of 0.5 $ml \cdot l^{-1}$.

2.2.2 *Feeding and Sampling Protocols*

Tables 2 and 3 outline the feeding, histological sampling and tagged fish measurement schedules for Experiments I and II, respectively. In Experiment I, fish were subjected to a 14-week restricted feeding regime in which a two week starvation period was followed by a two week period of excess feeding (2:2 regime). The first restricted feeding period commenced in November with Group 1 and ended in June with Group 5. For Experiment II a stricter feeding regime was implemented where fish were subjected to six week starvation periods (6:0 regime) with no intermittent feeding. Starvation periods in this experiment commenced earlier, running from September (Group 1) through April (Group 5).

Fish were sampled throughout both experiments to follow the normal process of gonadal development and to assess the effects of restricted feeding on growth, body condition and gonadal development. At the beginning of each experiment samples of fish were killed to establish a baseline measurement of gonadal development. In Experiment I, this sample consisted of 36 fish injured during transport. In Experiment II, a representative sample of 40 fish was selected and used for this measurement. Sample size at other measurement times, with the exception of the final samples, was ten fish per tank. All sampled fish were killed by overdose of anaesthetic, either MS222 or 2-phenoxyethanol, lengthed to the nearest 0.1 cm, weighed to the nearest gram, and dissected to remove the gonads. The gonads were macroscopically examined and classified for sex and maturity, weighed to the nearest $gram^{-4}$ and then immediately fixed in Bouin's solution for histological

Table 2: Experiment I. Restricted feeding and sampling schedule. *, sample taken for histology analysis; shaded blocks, starvation weeks; T, termination of group; S# tagged fish measurement times.

Month	Week beginning	Tagged measurements	Group					
			Control	1	2	3	4	5
Nov	4	S0	*					
	11							
	18							
	25		*					
Dec	2							
	9							
	16		*	*				
	23							
	30							
Jan	6	S1	*					
	13							
	20		*					
	27			*	*			
Feb	3							
	10		*					
	17							
	24					*		
Mar	3		*		*			
	10	S2		*				
	17							
	24		*					
	31						*	
Apr	7					*		
	14		*		*			
	21			*				
	28							
May	5	S3	*					*
	12						*	
	19					*		
	26		*		*			
Jun	2			*				
	9							
	16		*					*
	23	S4					*	
	30					*		
Jul	7		*		*			
	14			*				
	21							
	28	S5	*	T	T	T	T	T
Aug	18		*					
Sep	8		*					
	29		T					

Table 3: Experiment II. Restricted feeding and sampling schedule. *, sample taken for histology analysis; shaded blocks, starvation weeks; T, termination of group; S# tagged fish measurement times.

Month	Week beginning	Tagged Measurement	Group					
			Control	1	2	3	4	5
Sep	15	S0	*					
	22							
	29							
Oct	6							
	13							
	20							
	27	S1	*	*	*	*	*	*
Nov	3							
	10							
	17							
	24							
Dec	1							
	8	S2	*	*	*	*	*	*
	15							
	22							
	29							
Jan	5							
	12							
	19	S3	*	*	*	*	*	*
	26							
Feb	2							
	9							
	16							
	23							
Mar	2	S4	*	*	*	*	*	*
	9							
	16							
	23							
	30							
Apr	6							
	13	S5	*	*	*	*	*	*
	20							
	27							
May	4							
	11							
	18							
	25	S6	*	*	*	*	*	*
Jun	1							
	8							
	15							
	22							
	29							
Jul	6	S7	T	T	T	T	T	T

analysis. Condition factor (CF) and gonadosomatic index (GSI) were calculated by Equations 1 and 2 as follows:

$$\textbf{Condition Factor} = \frac{Weight}{Length} \times 100 \qquad (1)$$

$$\textbf{Gonadosomatic Index} = \frac{Gonad\ Weight}{Weight} \times 100 \qquad (2)$$

Tagged fish were monitored for length and weight periodically throughout each experiment. Condition factors were calculated as described above and as well, specific growth rates (SGR), based on changes in weight over time, were calculated between sampling periods according to Equation 3:

$$\textbf{Specific Growth Rate} = \frac{lnwt_2 - lnwt_1}{t_2 - t_1} \times 100 \qquad (3)$$

where $ln\ wt_1$ and $ln\ wt_2$ are the natural logarithms of the weights at times 1 and 2, respectively, and $t_2 - t_1$ is the number of days between sampling times.

At the termination of both experiments all fish were killed, measured and dissected. However, in Experiment I, only 80 fish from each control tank were killed along with the rest

of the groups. The remaining fish were kept to follow gonadal development until autumn. Sampling of these fish continued as scheduled until termination of the experiment on 2 October 1997, necessitated by the onset of PKD.

2.3 HISTOLOGY

Gonads removed from sampled fish were fixed and preserved in Bouin's solution (15 saturated picric acid: 5 formalin: 1 glacial acetic acid) until the time of histological examination. Whole testes of immature fish were fixed and used for examination; in maturing fish, only the anterior portion (~1/3) of one testis was kept for processing. Tissues selected for microscopic examination were dehydrated in ethanol, cleared in xylene and embedded in Paraplast Plus® paraffin. Blocks were trimmed and cut transversely at 5μm on a rotary microtome. Sections were floated on slides smeared with Mayer's glycerine-albumin for adhesion and dried for 24 hours at 40°C on a slide warmer. Sections were stained with Ehrlich's hematoxylin, counterstained with eosin Y and mounted with Permount™.

2.4 DATA ANALYSIS AND STATISTICS

2.4.1. *Final Maturation Proportions*

Differences in the proportions of fish maturing between treatment replicates and between the control and each experimental group were compared using a Generalized Linear

Model for the analysis of binomial frequencies. The format of the model used was:

$$f = e^{\beta} \bullet N + \epsilon$$

where f is the observed frequency, $e^{\beta} \cdot N$ is the expected frequency and ϵ is the residuals of the fit of the model to the data. The statistic used was the G-test, calculated using Log-Likelihood Ratios (Sokal and Rohlf 1995; Equations 17.1 and 17.3, p. 689-690). Tolerance for Type I error was set at 5%, and the observed p-value found using the Chi-square frequency distribution in the Minitab® statistical package (Minitab® Release 9.2 for Windows).

Differences between replicate tanks and between groups were conducted by binomial comparison of the proportions of fish identified as maturing (Sokal and Rohlf 1995; Chapter 17). Additionally, a binomial analysis fitting the treated groups directly to the control was designed as a more sensitive test for treatment effects. In this analysis, the control fish were taken as a fixed effect, rather than a random sample from a larger population. That is, the expected frequency to which the experimental groups were compared was taken to be the observed frequency of maturing males in the control group, rather than comparing how the groups and control deviate from an expected mean value. Although this approach is not ideal, since it bases the model on an expected frequency with inherent error, it is justified in

that the internal control group are the only fish that the treatment groups can be legitimately compared with for the detection of treatment effects.

2.4.2 *Growth and Performance*

2.4.2.1 Final Sampling Data

Final growth parameters of weight, length, condition factor and gonadosomatic index were based on individual measurements of all fish of a given sexual status (maturing male, immature male or female). Analysis of Variance (ANOVA) was used to test for growth differences between replicates and treatment groups, and for differences attributable to sexual state within groups. Tukey's pairwise comparisons with a family error rate of 0.05 were used to isolate differences between groups. For all tests, tolerance for Type I error, α, was set at 0.05. Residuals of the fit of the data to the models were tested for normality using histograms and normal probability plots. Data which did not meet the assumptions of normality were log-transformed and when necessary, randomized 5000 times to generate new p-values.

2.4.2.2 Tagged Fish

Analysis of data for tagged fish was conducted using individual fish measurements, adjusting retrospectively from the final sampling for sexual status. Analysis of Covariance (ANCOVA), with time as covariate, was used to compare slopes of growth parameters

between treatments for maturing and immature male fish. All data were log transformed to improve the fit of the data to a linear model. When the overall ANCOVA model revealed significant interactions between time and the other explanatory variable (either sex or treatment group), the model was discarded. Differences between groups were then assessed at each discrete measurement time by one-way ANOVA to determine when differences between treatment groups occurred. Tukey's pairwise comparisons were employed when it was of interest to know which groups deviated significantly from the others.

3.0 RESULTS

3.1 TESTICULAR MORPHOLOGY

In Arctic charr the testes are paired elongate structures situated dorsal to the gut and extending the full length of the coelomic cavity. They are physically attached to, and supported by, the swimbladder along their length by mesorchia arising from the peritoneum covering the swimbladder. Posteriorly, gonoducts leaving each testis unite to form a common duct which opens into the urogenital papilla. The testes are of the unrestricted type (Grier 1981; Billard 1986; Pudney 1995) where the tissues are organized as a system of lobules in which spermatogonia are distributed along the entire length. In the non-reproductive state, the lobules are composed of primary spermatogonia, secondary spermatogonia, and Sertoli cells. Extra-lobular material is made up of Leydig cells and connective-type tissues. With the onset of spermatogenesis, spermatogonia rapidly proliferate, lobular lumens, into which spermatozoa are eventually released, form and cysts of germ cells develop.

3.2 Stages of Spermatogenesis

Spermatogenic stages were classified according to the criteria described below. This classification, although derived specifically from observations made in this study, is comparable to previous descriptions of teleost testicular development described by Henderson (1962) and Grier (1981).

Stage I. *Pre-spermatogonial*

Macroscopically, testes at this stage of development are barely identifiable as distinct organs. They are thin, transparent, thread-like structures running parallel along the swimbladder. Typically, they are associated with a gonadosomatic index (GSI) of less than 0.05. Microscopically, neither testicular lobules nor spermatogonia are distinguishable. The tissue consists simply of a mass of diffuse connective-type tissue, and some interstitial cells that stain darkly and appear to be Leydig cells (Figure 2).

Stage IIa. *Immature I*

Testes of fish classified as immature are more advanced than pre-spermatogonial individuals in that, macroscopically, the testes display an obvious increase in width. They remain transparent at this stage, but have characteristic pinkish hues not detectable at the earlier stage, a possible consequence of increased vascularization. GSI values are approximately 0.05. Under light microscopy, the lobular structure of the testes is

distinguishable, but at this stage individual lobules are small and per section, usually contain only one to a few "A" type, or primary spermatogonia (Figure 3). This type of spermatogonium is recognized by its large size, distinct cytoplasm and central, round nucleus containing usually a single nucleolus and several chromatin patches which stain darkly with hematoxylin. The tissue is very compact as lobular lumens have not yet formed. Mitotically dividing spermatogonia can be identified but their occurrence is rare indicating that the tissue is in a slow phase of spermatogonial proliferation.

Stage IIb. ***Immature II***

This stage of spermatogenesis is distinguished from the previous by a further increase in width of a transparent testis and GSI values typically approaching 0.10. At this stage, the testis does not increase uniformly in width along its length, resulting in a convoluting, irregular shaped structure. Under light microscopy, the development of the tissue is obvious by the increase in the number of spermatogonia at various stages of mitotic division. This proliferation of spermatogonia results in there being an increased number of germinal cells per lobular section. Most spermatogonia are still "A" type, but numerous "B" type can be discriminated. "B" type spermatogonia are slightly smaller than primary cells as their cytoplasmic and nuclear content is reduced. Their nuclear structure also differs in that there are usually two or three nucleoli present and the chromatin patches disperse giving the

nucleus a darker and more uniform appearance. Lumens begin to open within the lobules decreasing the density of the tissue (Figure 4).

Stage III. ***Transient***

Fish at this stage of spermatogenesis show drastic increases in the level of mitotic activity of the spermatogonia. Spermatogonia can now be identified dividing as a group, making cysts of cells readily distinguishable (Figure 5). Most spermatogonia at this stage are "B" type, and even further reduced in size from having undergone repeated divisions. Primary cells are still identifiable, but are fewer in number and seem to be restricted to the boundaries of the lobules. Lobular lumens widen and become more distinct. Further irregular increases in testicular width are noticeable macroscopically, however, the testis remains translucent and little change in GSI value (ca. 0.10) is observed from the previous stage.

Stage IVa. ***Spermatocyte Formation I***

This stage is distinguished by the appearance of primary spermatocytes which are initially recognizable by the aggregation of the chromatin into one pole within a nucleus (Figure 6). The transformation from spermatogonia "B" into primary spermatocytes occurs synchronously in all cells within a cyst, and is not associated with any change in cell size. The differences between these cells are restricted to nuclear structure and appearance. In

primary spermatocytes, the nucleoli disappear and the cells undergo the first maturation division of meiosis. At this point the testes undergo rapid increases in size, GSI values rise quickly beyond 0.10, usually to values above 1.0. The testes are no longer transparent, they become increasingly opaque, but continue to retain their pinkish coloration. This stage can only be identified during the period of time extending from March until June.

Stage IVb. ***Spermatocyte Formation II***

This stage is characterized by the appearance of secondary spermatocytes, the end products of the first meiotic division (Figure 7). These cells resemble their precursors in appearance, but are noticeably smaller. Lobular lumens are no longer very distinct as the expanding cysts crowd into the space. Macroscopically, the testis is similar to the previous stage except that size and GSI are still increasing.

Stage V. ***Spermatid Formation***

This stage is marked by the formation of spermatids, the products of the second meiotic division. Spermatids are distinguished from secondary spermatocytes by a further reduction in size and the distinctly round shape of the darkly staining nuclei (Figure 8). The testes continue to increase in size and GSI value, and become increasingly opaque.

Stage VI. ***Spermatozoa***

This stage is characterized macroscopically by distinct changes in the coloration of the testes. As this stage progresses, bands of white coloration form along the testes corresponding to the transformation of spermatids into spermatozoa. This transformation is recognized by the concentration of the nuclear material into one pole of the nucleus and the addition of a flagellum to each of the cells. As they are produced, spermatozoa are released from their cysts into the lumen of the testicular lobule and are seen as dark concentrations of sperm heads (Figure 9). Primary spermatogonia can still be observed along the boundaries of the lobule, supposedly acting as a reservoir of germinal cells for subsequent reproductive cycles. GSI and testicular size continue to increase until the entire testis is composed of spermatozoa lying free within the lobules.

Stage VII. ***Functional Maturity***

As spermatozoa formation continues, the testes become progressively composed of spermatozoa lying free in the lumens of the lobules (Figure 10). At this stage, the testes are milky white and sperm readily discharges when pressure is applied to the testes.

The classification system as described above is summarized in Table 4. It is important to note that each of the described stages does not occur synchronously in all fish of a population. Although the length and prevalence of the different stages varies greatly

with individuals, an attempt is made herein to describe the basic time frame for the various stages of development (Table 5). In addition, although cells of an individual cyst develop synchronously, the testes of an individual fish usually contain cysts with characteristics of more than one stage (Figure 11). Therefore, for this study, individual fish were classified based on the stage of development which was most prevalent in the histological sections.

Several fish in this study displayed partial development of the testis, complete to the formation of spermatozoa in which it appeared that only a few cysts proceeded through the maturation cycle while the rest of the testis remained non-reproductive (Figure 12). As this state of development was observed in September samples, it is unlikely that these were fish which were just commencing development. The absence of any of the intermediate, spermatocyte or spermatid, stages supports this. These fish were considered non-reproductive as it is unlikely that such a reduced state of development could ever constitute a functionally mature male. In addition, these fish did not display significantly increased GSI values, which lends support to the idea that such development was minor. It is possible that these fish represent individuals in which gonadal development which had begun was subsequently arrested, and those cysts which had proceeded to develop beyond a certain critical point (Stage IV) were compelled to produce spermatozoa.

A small percentage (approximately 3%) of the fish were already reproductive at the beginning of both experiments. Amongst these fish, those that underwent a subsequent

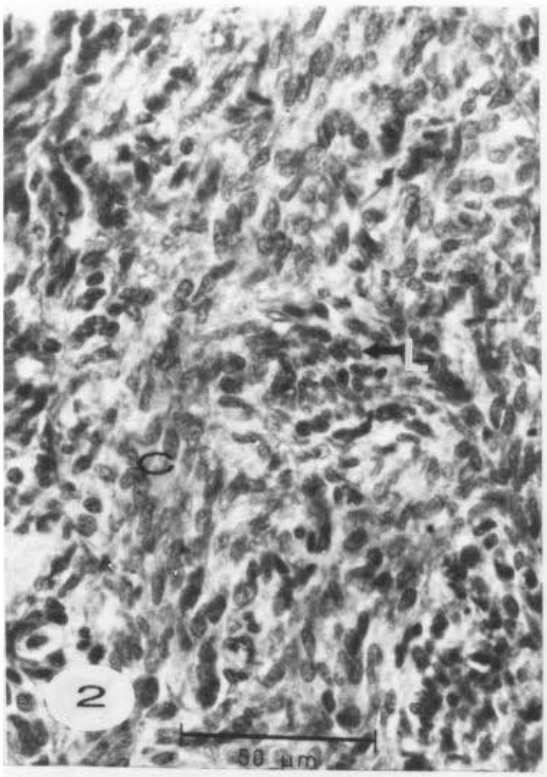
maturation progressed through similar stages of spermatogenic activity as those observed for virgin fish. Gonadal recrudescence began by proliferation of the primary spermatogonia in the lobules and continued to progress through the stages of development while concurrently, residual spermatozoa from the previous cycle were phagocytosed. It was observed that the complete resorption of spermatozoa remaining from a previous cycle can take up to six months or longer as residual sperm could be identified in testis up until June of the following year (Figure 13). Fish undergoing a repetitive maturation cycle are easily distinguished from virgin fish in that the testes are much enlarged and have a white to grey color depending on the degree of residual sperm resorption. Microscopically, previously reproductive fish are distinguished from those fish showing incomplete maturation by the enlarged testicular lumens in the former (See Figures 12 and 13).

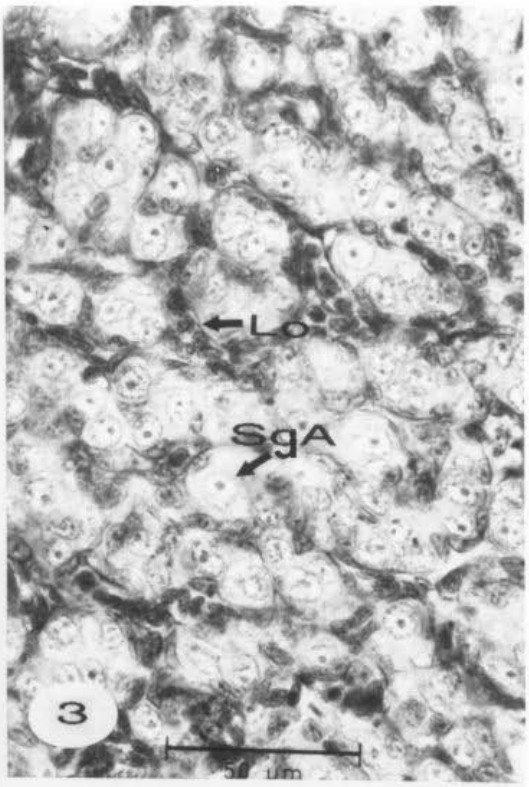
Figure 2: Testis of a completely immature fish at Stage I, *Pre-spermatogonial.* No spermatogonia or lobular structure is visible. L, Leydig cells; C, connective tissue. Bar = 50 μm.

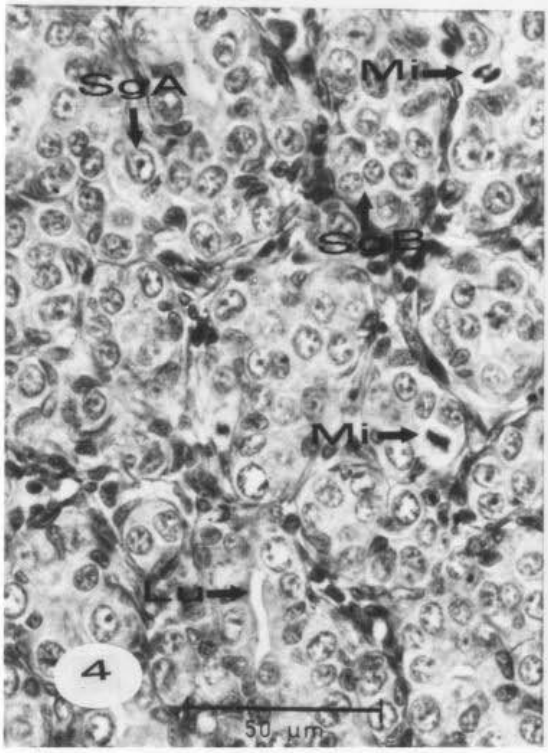
Figure 3: Testis of a fish at Stage IIa, *Immature I*, of development. Lobular structure is defined and primary spermatogonia, recognizable by the large central nucleolus, are present either singly or few per lobule. Lo, lobule wall or boundary; SgA primary spermatogonium. Bar = 50 μm.

Figure 4: Testis of a fish at Stage IIb, *Immature II* of development. Lobules contain greater numbers of spermatogonia with both primary and secondary types present. Mitotic proliferation of spermatogonia is observable and lumens open within the lobules. SgA, primary spermatogonium; SgB, secondary spermatogonia; Mi, spermatogonium undergoing mitotic division; Lu, lumen. Bar = 50 μm.

Figure 5: Fish testis at Stage III, *Developing*. Lobules are large and contain primarily secondary spermatogonia. Drastic increases in the number of mitotically dividing cells are seen and cells begin to divide as a cyst. SgA, primary spermatogonium; Mi, mitosis occurring singly in a spermatogonium; MiG, group mitoses occurring simultaneously in cells within a cyst. Bar = 50 μm.


L
C
2
50 µm


Lo
SgA
3


SgA
Mi
SgB
Mi
L
4
50 µm

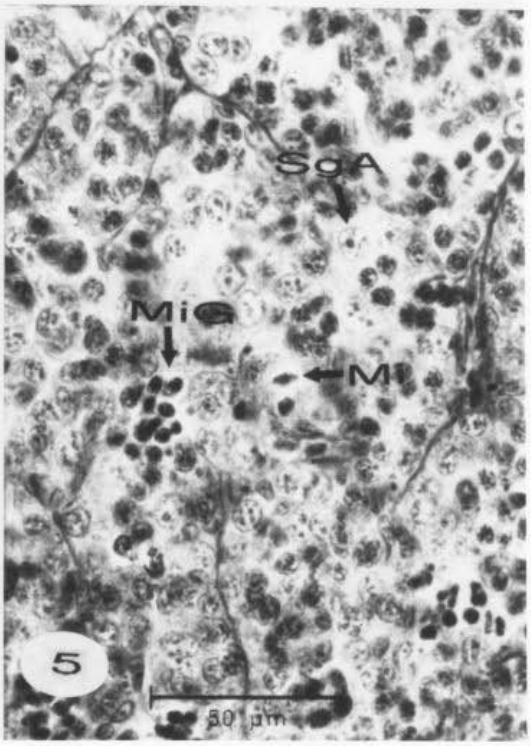
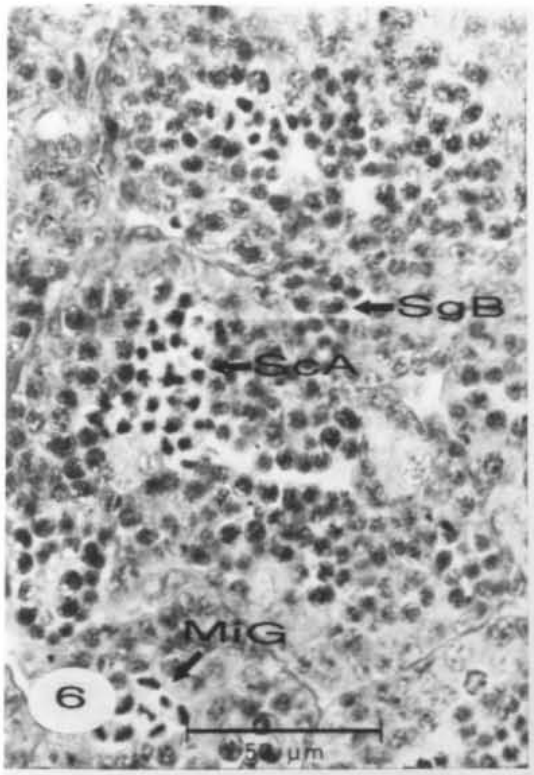

SgA
Mi
Mi
5
50 µm

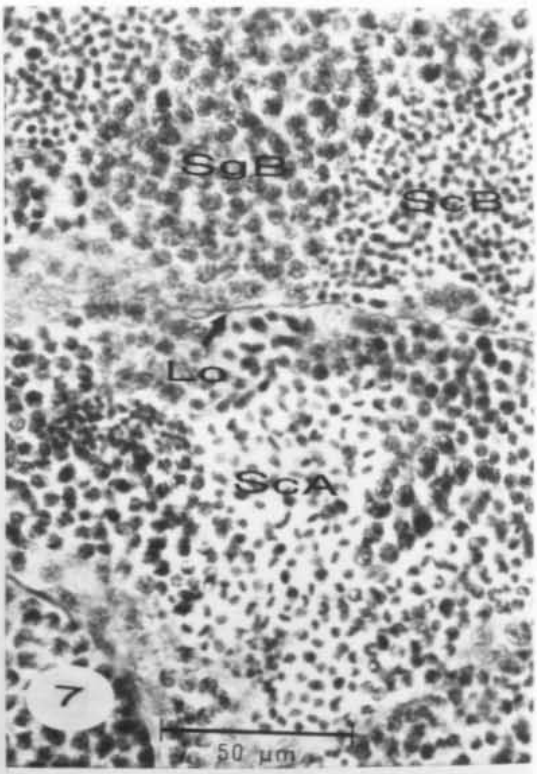
Figure 6: Testis at initial stage of spermatocyte formation and meiotic division, Stage IVa, *Spermatocyte Formation* I. SgB, secondary spermatogonium; ScA, primary spermatocytes; MiG, group mitoses. Bar = 50 μm.

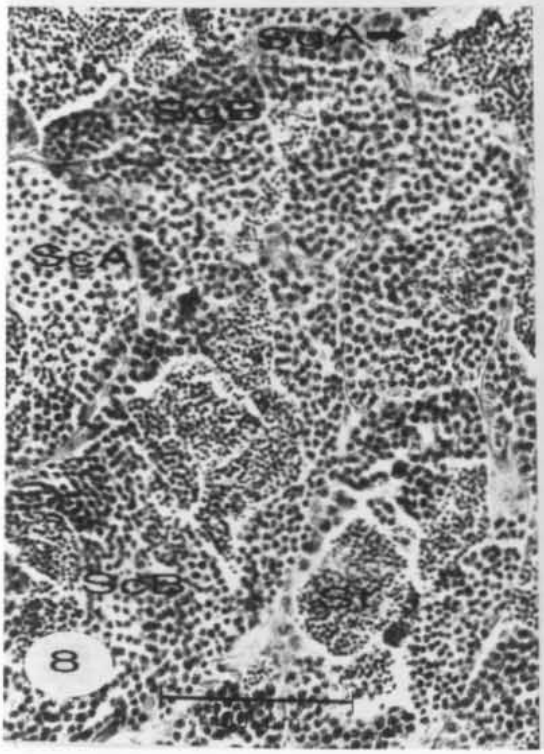
Figure 7: Testis showing second stage of spermatocyte formation, Stage IVb, *Spermatocyte Formation II.* Primary spermatocytes and secondary spermatocytes are observable within testicular lobules. SgB, secondary spermatogonia; ScA, primary spermatocytes; ScB, secondary spermatocytes; Lo, lobular wall. Bar = 50 μm.

Figure 8: Testis at *Spermatid Formation* stage of development, Stage V. SgA, primary spermatogonium; SgB, secondary spermatogonia; ScA, primary spermatocytes; ScB, secondary spermatocytes; St, spermatids. *Note*: magnification is lower, Bar = 100 μm.

Figure 9: Testis advanced to *Spermatozoa* stage of development, Stage VI. Spermatozoa are recognized by the darkly staining sperm heads and the presence of tails. SgA, primary spermatogonium; SgB, secondary spermatogonia; ScA, primary spermatocytes; St, spermatids Sz, spermatozoa; T, sperm tails. Bar = 50 μm.


SgB
ScA
MiG
6
50 µm


SgB
ScB
Lo
ScA
7
50 µm


SgA
SgB
ScA
ScB
8

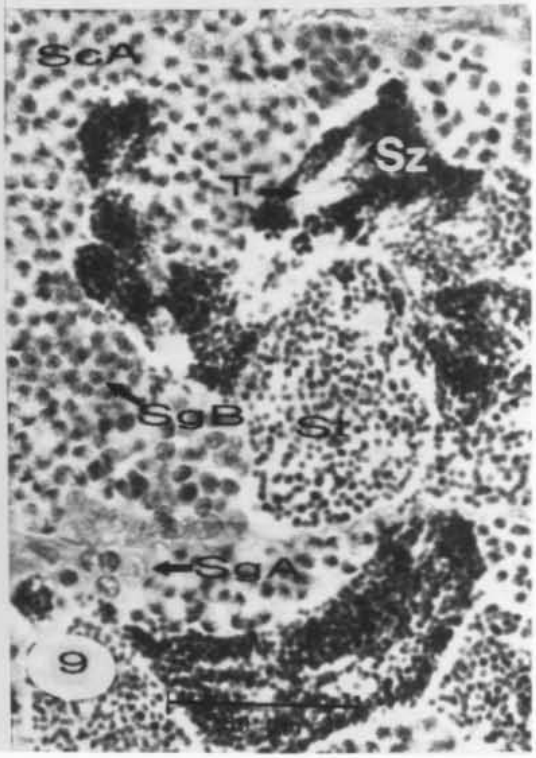
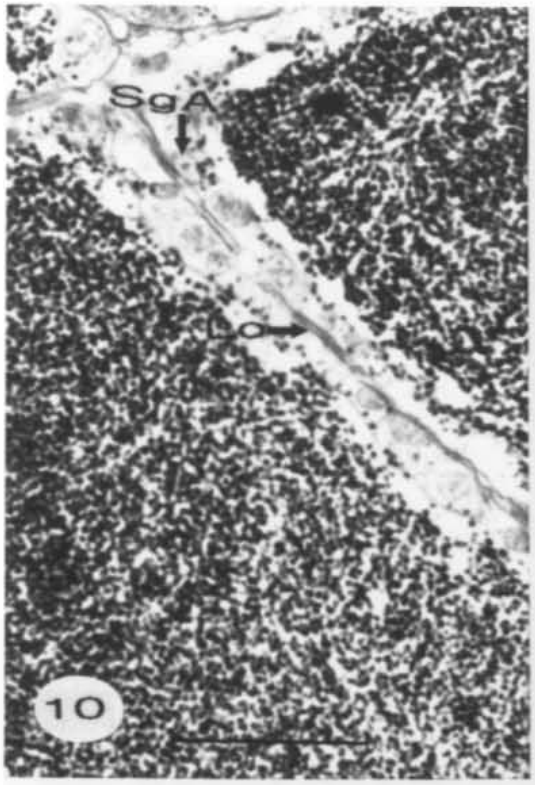

ScA
Sz
T
SgB
St
SgA
9

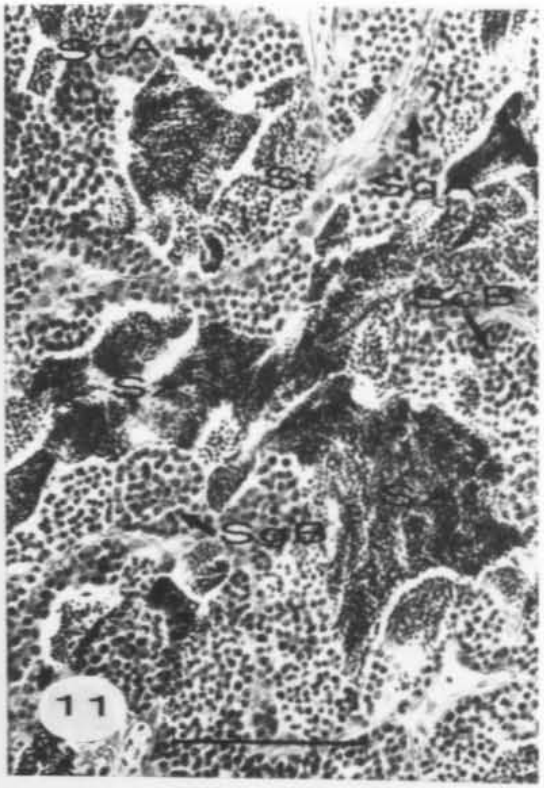
Figure 10: Fish at stage of *Functional Maturity*, Stage VII. Testis consists of darkly staining spermatozoa lying free in the lumens of lobules. Primary spermatogonia line the lobule walls. SgA, primary spermatogonium; Lo, lobular wall. Bar = 50 μm.

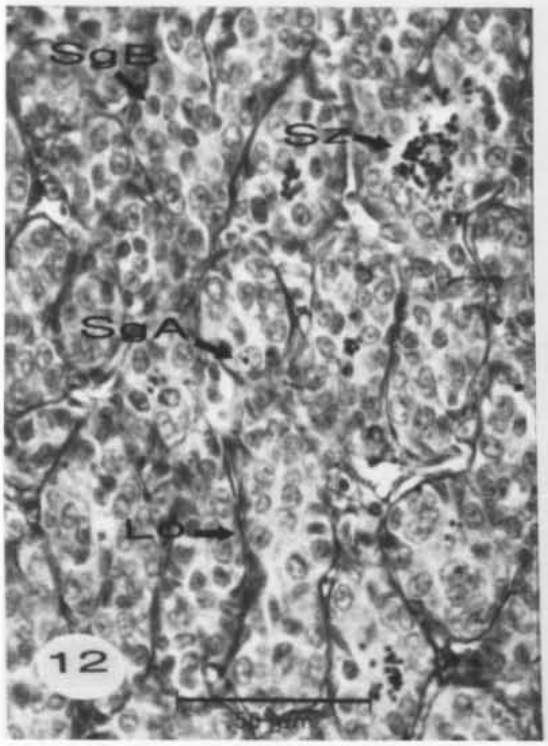
Figure 11: Testis showing asynchrony of lobular progression of spermatogenic stages. Cells within a cyst proceed through development together but not all cysts are synchronous within the lobule. SgA, primary spermatogonium; SgB, secondary spermatogonia; ScA, primary spermatocytes; ScB, secondary spermatocytes; St, spermatids; Sz, spermatozoa. *Note*: magnification is lower. Bar = 100 μm.

Figure 12: Fish testis (September sample) showing an aborted or incomplete maturation. Only a few spermatozoa are present in the lumens of some lobules amongst spermatogonia. SgA, primary spermatogonium; SgB, secondary spermatogonia Sz, spermatozoa; Lo, lobular wall. Bar = 50 μm.

Figure 13: Testis (June sample) of previously mature fish showing resorption of residual spermatozoa remaining from the previous spawning season. Primary spermatogonia line the lobule walls. Rz, residual spermatozoa undergoing phagocytosis. SgA, primary spermatogonium; Mi SgA, mitotically dividing spermatogonium, Lo, lobular wall, Lu, lumen. Bar = 50 μm.


SgA
Lo
10


SgA
SgB
11


SgB
Sz
SgA
Lo
12

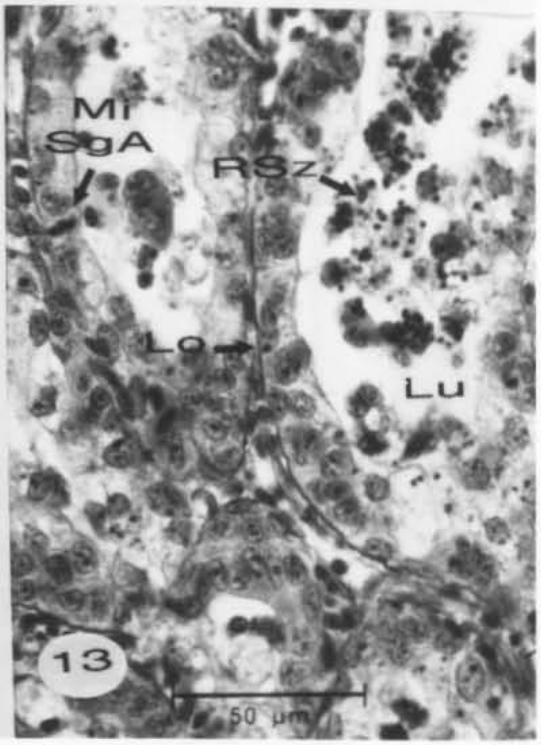

Mi
SgA
RSz
Lo
Lu
13
50 µm

Table 4: Summary of the microscopic characteristics distinguishing the spermatogenic stages of Arctic charr, *Salvelinus alpinus*. +, indicates the relative proportions of the various characteristics; -, indicates absence of the characteristic; ±, may or may not be identifiable.

Stage	Name	Spermatogonia		Spermatocytes		Spermatids	Spermatozoa	Mitosis	Meiosis	Lumens
		A	B	A	B					
I	Pre-spermatogonial	±	-	-	-	-	-	-	-	-
IIa	Immature I	++	-	-	-	-	-	+	-	-
IIb	Immature II	+++	+	-	-	-	-	++	-	+
III	Transient	++	+++	-	-	-	-	+++	-	+++
IVa	Spermatocyte Formation I	+	+++	++	-	-	-	++	+	++
IVb	Spermatocyte Formation II	+	++	+++	++	-	-	+	+++	++
V	Spermatid Formation	+	+	++	+++	++	-	+	+++	+
VI	Spermatozoa	+	±	+	+++	+++	++	+	++	++
VII	Functional Maturity	+	-	-	-	+	+++ (lumen)	±	-	+++

Table 5: Timing and duration of the spermatogenic stages of Arctic charr, *Salvelinus alpinus*. +, indicates the relative proportions of the various characteristics; -, indicates absence of the characteristic; ±, may or may not be identifiable; r, residual spermatozoa may be present.

Stage	Name	Month											
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept
I	Pre-spermatogonial	+++	++	+	+	+	+	+	+	+	+	+	+
IIa	Immature I	+	+++	++	+	+	+	+	+	+	+	+	+
IIb	Immature II	+	+	++	+++	+	+	+	+	+	+	+	+
III	Transient	-	-	-	+	++	+++	+++	++	+	-	-	-
IVa	Spermatocyte Formation I	-	-	-	-	-	+	++	+++	+++	-	-	-
IVb	Spermatocyte Formation II	-	-	-	-	-	-	+	++	+++	+++	++	-
V	Spermatid Formation	-	-	-	-	-	-	-	+	++	+++	+++	+
VI	Spermatozoa	++	++	+	r	r	r	r	r	r,+	++	+++	+++
VII	Functional Maturity	+++	++	+	-	-	-	-	-	-	++	+++	+++

3.3 Effects of Starvation on Spermatogenesis and Maturation

3.3.1 *Final Numbers of Fish and Survival*

The numbers of fish in the final samples, separated according to sexual status, are shown in Table 6. Survival of fish during both experiments was very high. During Experiment I, an accidental loss of 38 fish from one control tank occurred as a result of a standpipe problem. No natural mortalities occurred during any of the starvation periods in either the first or second experiment, indicating that the restriction periods were not overly severe. When temperatures increased in the spring, there was an insignificant loss of approximately one fish per day. These losses occurred in fish that were in poor condition. Although the fish were diagnosed with PKD, during the actual experimental periods, no losses were attributable to this parasitic infestation. The presence of this disease was only recognized in those fish which were held beyond the termination of Experiment I. These fish were not used in any quantitative analyses.

Table 6: Numbers of fish at the termination of both Experiments I and II.

Sex	Experiment I				Experiment II			
	N		%		N		%	
Maturing males	354	∑♂	39.7	∑♂	291	∑♂	28.8	∑♂
Immature males	158	512	17.8	57.5	308	599	30.5	59.3
Females	379		42.5		412		40.7	
Total	891		100		1011		100	

3.3.2 *Starvation Effects on Spermatogenesis*

The staggered sampling schedule used throughout Experiment I did not permit direct comparisons of the effects of starvation on the spermatogenic cycle to be made between the groups during the experiment. However, sampling throughout the experimental period in Experiment II, which was all conducted on the same day, made such comparisons feasible. The characteristics that were used at the different sampling times to assign fish into categories where testicular development was considered to be ongoing are described in Table 7. Table 8 and Figure 14 show the results of this sampling.

At each sampling time during the periods of starvation (September to April), the group which exhibited the lowest proportion of fish with signs of testicular development was always that group which was starved in the six weeks previous to sampling. The probability that the starved group, out of a possible 6 groups, always ended up in this position by chance

Table 7: Histological characteristics and stages of development defining testicular development as used in classifying sampled fish over the time period of Experiment II.

Time	Stage(s)	Characteristics of testes showing testicular development
Sep 15	II, III	Spermatogonia A, and/or B obvious, and in lobules
Oct 27	II, III	Spermatogonia A, and/or B obvious, and in lobules
Dec 8	II, III	Spermatogonia A, and/or B obvious, and in lobules, mitosis clearly evident
Jan 19	III	Spermatogonia A, and B obvious,increased number cells / lobule, lumens opening, mitosis clearly evident
Mar 3	III, IV	Spermatogonia A, and B obvious,increased number cells / lobule, lumens opening, mitosis, or cyst formation with spermatocytes A or B
Apr 23	III, IV	Spermatogonia A, and B obvious,increased number cells / lobule, lumens opening, mitosis, or, Spermatocytes A, and B or any further advanced state
May 5	III - VII	Spermatogonia A, and B obvious,increased number cells / lobule, lumens opening, mitosis, or, Spermatocytes A, and B or any further advanced state
Jul 3	IV - VII	At least at spermatocyte stage, or any advanced stage to spermatozoa

* Stage I, *Pre-spermatogonial*, fish is not considered to be showing testicular development

Table 8: Numbers and proportions of virgin male fish showing signs of active spermatogenesis during Experiment II. D = number of virgin male fish exhibiting testicular development, T = total number of virgin male fish in sample, P = percentage exhibiting spermatogenesis and testicular development. Numbers in bold represent the group showing the lowest proportion of fish showing testicular development for the sampling period.

	Sept 15			Oct 27			Dec 8			Jan 19			Mar 2			Apr 13			May 25			Jul 6		
	D	T	P	D	T	P	D	T	P	D	T	P	D	T	P	D	T	P	D	T	P	D	T	P
Initial	5	21	23.8																					
Control				2	7	28.6	5	9	55.5	3	9	33.3	5	7	71.4	5	8	62.5	8	15	53.3	51	90	56.7
Group 1				2	14	**14.3**	3	11	27.3	5	15	33.3	5	10	50	6	12	50	4	10	40	42	97	**43.3**
Group 2				4	12	33.3	3	13	**23.1**	4	11	36.4	7	12	58.3	5	9	55.5	8	17	47.1	57	112	50.9
Group 3				5	12	41.7	8	14	57.1	1	9	**11.1**	3	9	33.3	5	9	55.5	5	10	50	49	102	48
Group 4				5	12	41.7	4	10	40	4	12	33.3	3	10	**30**	5	7	71.4	7	14	50	47	96	49
Group 5				5	13	38.5	6	11	54.5	4	13	30.8	5	12	41.7	6	14	**42.9**	2	8	**25**	45	101	44.6

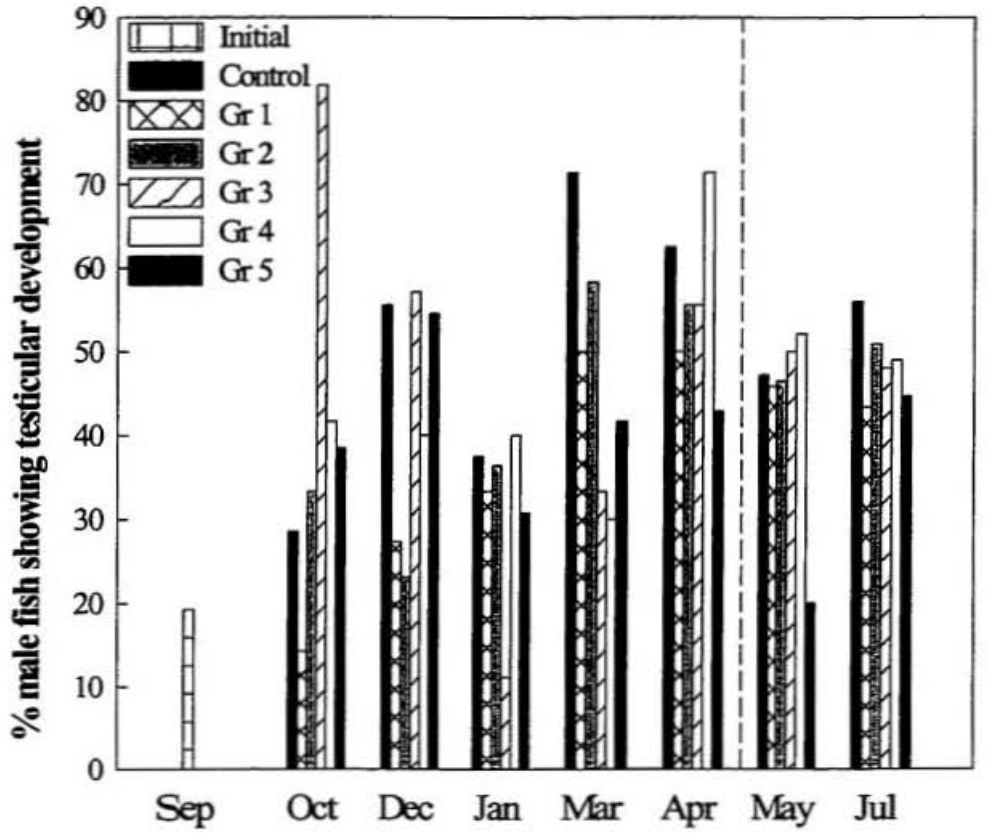


Figure 14: Percentages of male fish showing signs of testicular development during Experiment II. Testicular development at each sampling time was as defined in Table 7. Dashed vertical line indicates the end of the starvation periods.

alone in five independent sampling events is 1 / 7776, or 0.000129 ($p < 0.001$) (Considering this as a binomial response variable with 6 classes then the probability $= (1/6)^5$; using a Chi-square distribution for computation of the associated p-value). This significant result suggests that starvation is capable of directly affecting testicular development. With the exception of Group 5, starved between 3 March and 13 April, all groups were able to catch up in gonadal development by the next sampling time. Between the April and May samples, Group 5 did not seem to resume gonadal development. Re-feeding of this group corresponded with increasing temperatures which may have made this a metabolically difficult catch-up situation. By the final sample in July, however, gonadal development was again underway.

3.3.3 *Critical Points of the Spermatogenic Cycle*

Histological observations throughout both experiments permitted the possible identification of two critical points in the spermatogenic cycle of Arctic charr where fish may prove to be susceptible to nutritional control of maturation. This supposition stems from the recognition that only two stages of spermatogenesis may be consistently identified at every sampling point throughout the year. These are the pre-spermatogonial and immature stages (Stages I and II). All other stages can be considered maturational stages and as such, are observed only temporarily during the yearly cycle. It appears from these observations that control factors acting to arrest gonadal development during the spermatogenic cycle must

occur as fish move from these stages into the next. That is, one critical point would occur as the fish advance from the pre-spermatogonial stage into the immature stage and a second as the fish move from the immature stage through the transient stage and into spermatocyte production. Otherwise, one would expect to see testicular development arrested at all spermatogenic stages.

Starvation of fish for six weeks in the period of mid-September through October during Experiment II was found to delay early gonadal development by preventing fish from advancing from a pre-spermatogonial stage into an immature stage. The initial sample of fish for Experiment II, taken on 15 September 1997, consisted of 26 males (26 males / 40 fish = 65%) of which two were functionally mature for the current spawning season. Of the remaining male fish, 19 (79.2%) had GSI values less than 0.05 and by both macro- and microscopic analysis were designated as being in the pre-spermatogonial stage of testicular development. The remaining 5 virgin fish (20.8%) had GSI values in the range of 0.05 to 0.08 and were classified to Stage II as they were considered to be showing signs of testicular development (Table 8).

The second sampling of fish for observation of gonadal development occurred on 27 October 1998, immediately subsequent to the first starvation period. At this time, the gonadal histology revealed that an advancement of testicular development occurred in fish fed during this period. Most fish were classified to Stages II and III. This effect was not seen in Group 1 fish which were starved during this time period. In this group, most fish

held a Stage I classification as only 14.3% were classified to later developmental stages. As this is an even lower proportion than the initial sample, it seems that these fish did not undergo any testicular development during this period as had their fed counterparts. This inhibition of development, however, proved only to be a temporary effect. Sampling at time 2, 16 December 1998, after these fish had been re-fed for six weeks, revealed that the observed delay in testicular development was no longer obvious. Although fish starved at other times did show delays in testicular development, in that there seemed to be a slowing in the rate of development during the period of food deprivation, at no other time was a delay in movement from one stage to the next apparent as it was during this first starvation period. The advancement of fish from the pre-spermatogonial state to that described as an immature stage thus appears to be a critical point in the gametogenic cycle which occurs in autumn. Although this stage seems to be affected by restricting food intake, it has shown to be somewhat flexible in its timing as resumption of feeding allowed a catch up in testicular development to occur in the following weeks.

The second point in the spermatogenic cycle which may act as a critical decision time occurs after Stage III, where fish proceed from the immature stages through the transient stage and into spermatocyte formation (Stage IV). Histological observations indicate, that once secondary spermatogonia begin dividing as a cyst and produce spermatocytes, they continue through the remaining spermatogenic stages until spermatozoa formation is complete. This is in concurrence with Billard (1986) who stated that

spermatogonia, once grouped in cysts, appear to be irreversibly engaged in spermatogenesis. If this were not the case, fish with various stages of cyst formation would be identifiable year round. That is, the fish would be able to arrest gonadal development at spermatocyte or spermatid stages of development. This clearly does not occur, indicating once cyst formation has been initiated, the cells will proceed through spermatogenesis to the production of spermatozoa. Cyst formation stages can be first identified in March in some fish, but the timing of this period appears to be very flexible as most fish do not reach this and later stages until at least May.

3.3.4 *Final Maturation Proportions*

Replicate treatment tanks in both experiments did not show significantly different maturation proportions at the termination of the experiments (See Appendix Tables A-1, A-2) so the replicate tanks were pooled as groups for all further analysis of maturation proportions ($p > 0.05$). Final maturation proportions for Experiment I ranged from 61.3 to 77.0 percent (Table 9; Figure 15A). Comparing the proportions of maturing fish in the treatment groups directly to the control group using the fitted binomial model showed that the proportions of fish maturing in Groups 2 and 3 were significantly different from the control. The observed difference is a result of there being a higher proportion of fish maturing in each of these groups than in the control. Groups 2 showed an 11.3% increase in the proportion of maturing fish over the control, and Group 3 was 13% higher. Using the

Table 9: Final maturation proportions of the control and experimental groups of fish in Experiment I. The control group was used to establish the expected ratio of mature to immature fish for the likelihood ratio test (G-statistic) for the control fitted binomial test. P-values determined using a Chi-square distribution with df = 1. Numbers in parentheses indicate the relative increase / reduction in maturation proportions of the treatment groups from the control.

Group	Number of Observations			Proportions		Control Fitted Binomial			Standard Binomial		
	Mature	Immature	Total	Mature	Immature	G - test	p -value	Sig. $\alpha = 0.05$	G - test	p -value	Sig. $\alpha = 0.05$
Control	57	32	89	64.0	36.0	-	-	-	-	-	-
1	59	27	86	68.6 (+4.6)	31.4	0.7925	0.3733	NS	0.4073	0.5233	NS
2	70	23	93	75.3 (+11.3)	24.7	5.3940	0.0202	S	2.2724	0.0988	NS
3	67	20	87	77.0 (+13.0)	23.0	6.8145	0.0090	S	3.5789	0.0585	NS
4	55	27	82	67.1 (+3.1)	32.9	0.3309	0.5651	NS	0.1733	0.6772	NS
5	46	29	75	61.3 (-2.7)	38.7	0.2307	0.6264	NS	0.1280	0.7205	NS

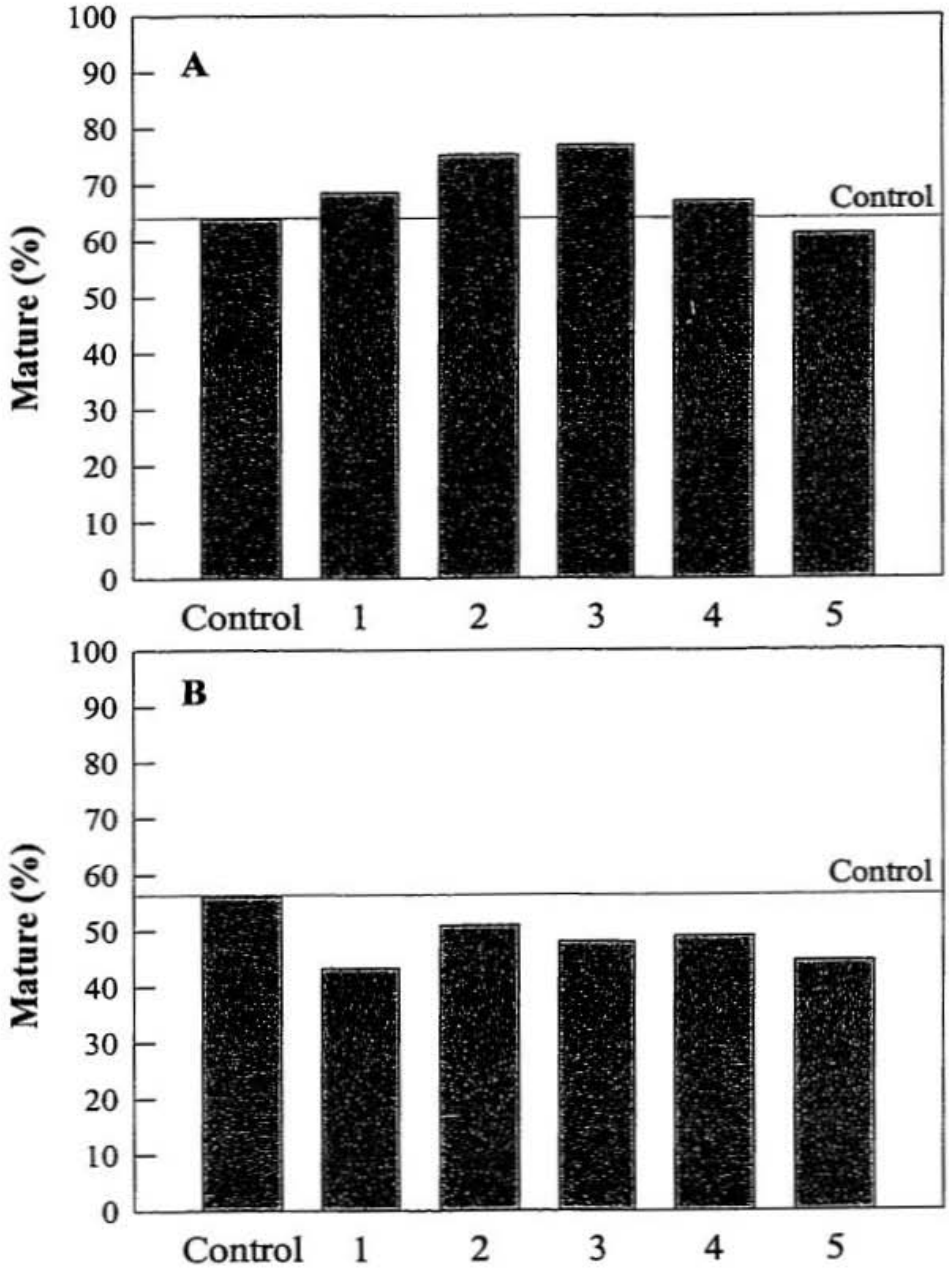


Figure 15: Final (July) proportions of maturing male fish in the control and feed restricted groups for A) Experiment I, and B) Experiment II.

standard binomial model, however, this difference was not detectable. There was no evidence that any of the 2:2 feeding regimes used in this experiment reduced the incidence of maturity. In effect, the opposite occurred, as the control group, which was fed throughout, exhibited one of the lowest proportions (64%) of maturing fish. Only Group 5, with a final proportion of 61.3%, was lower, but this reduction (2.7%) was not statistically significant. It is likely that the feeding regime used in this study actually promoted maturation by stimulating feed restricted fish to eat more when intermittent meals were provided between starvation weeks.

The final maturation proportions for Experiment II were lower than those of Experiment I (Table 10; Figure 15B). The proportions of fish that matured ranged from 43.3% in Group 1 to 56.7% for the control group. In this experiment, the 6:0 feeding regime appears to have reduced the incidence of maturity in comparison to continually fed fish. However, only the reductions observed in Groups 1 (-12.7%) and 5 (-11.4%) deviated significantly from the control group when using the fitted binomial model. Again, the standard binomial model did not detect this difference. These reductions, however, may not be due to reduced feeding at a specific time, but to the effect of starvation at times of higher temperature (Figure 1) in comparison to the other groups. It is interesting to note that the starvation times of these groups correspond well with the histological observations that a promotion of gonadal recrudescence seems to occur in autumn and in late spring as discussed earlier.

Table 10: Final maturation proportions of the control and experimental groups of fish in Experiment II. The control group was used to establish the expected ratio of mature to immature fish for the likelihood ratio test (G-statistic) for the control fitted binomial test. P-values were determined using a Chi-square distribution with df = 1. Numbers in parentheses indicate the relative increase / reduction in maturation proportions of the treatment groups from the control.

Group	Number of Observations			Proportions		Control Fitted Binomial			Standard Binomial		
	Mature	Immature	Total	Mature	Immature	G - test	p -value	Sig. $\alpha = 0.05$	G - test	p -value	Sig. $\alpha = 0.05$
Control	51	40	91	56.0	44.0	-	-	-	-	-	-
1	42	55	97	43.3 (-12.7)	56.7	7.0102	0.0081	S	3.0592	0.0803	NS
2	57	55	112	50.9 (-5.1)	49.1	1.5258	0.2167	NS	0.5356	0.4643	NS
3	49	53	102	48.0 (-8.0)	52.0	3.0835	0.0791	NS	1.2359	0.2663	NS
4	47	49	96	49.0 (-7.0)	51.0	2.3204	0.0684	NS	0.9413	0.3319	NS
5	45	56	101	44.6 (-11.4)	55.4	5.9963	0.0143	S	2.5333	0.1115	NS

3.4 Gonadal Tissue Investment

Mean gonadosomatic indices of the fish from the final sampling in July of both experiments show that no females exhibited showed any signs of maturation; the mean GSI values were 0.258 ± 0.016 and 0.232 ± 0.005 for Experiments I and II, respectively (Figure 16).

The GSI values of maturing males were typically greater than 0.010; immature fish most often lay in the range of 0.01 to < 0.10. However, this division cannot be taken as definitive as GSIs in the range of 0.10 to 0.20% are not always exclusive to maturing fish, necessitating histological examination of testes for correct classification of sexual status. The mean GSI values of maturing males was 3.5 ± 0.1 (Experiment I) and 3.8 ± 0.1 (Experiment II). Immature fish had mean values of 0.047 ± 0.002 (Experiment I) and 0.059 ± 0.003 (Experiment II).

Analysis of Variance of the final (July) GSI values indicated that restricted feeding, at any time, did not have any significant effects on the extent of gonadal investment for females, maturing or immature males between groups in either Experiment I (females: $F_{.05[5,374]} = 1.59$, $p = 0.163$; maturing males: $F_{.05[5,349]} = 2.10$, $p = 0.065$; immature males: $F_{.05[5,153]} = 1.33$, $p = 0.254$) or Experiment II (females: $F_{.05[5,407]} = 0.56$, $p = 0.733$; maturing males: $F_{.05[5,286]} = 0.65$, $p = 0.664$, immature males: $F_{.05[5,303]} = 1.35$, $p = 0.242$). It is likely that the restricted feeding periods were not severe enough to cause lasting reductions in

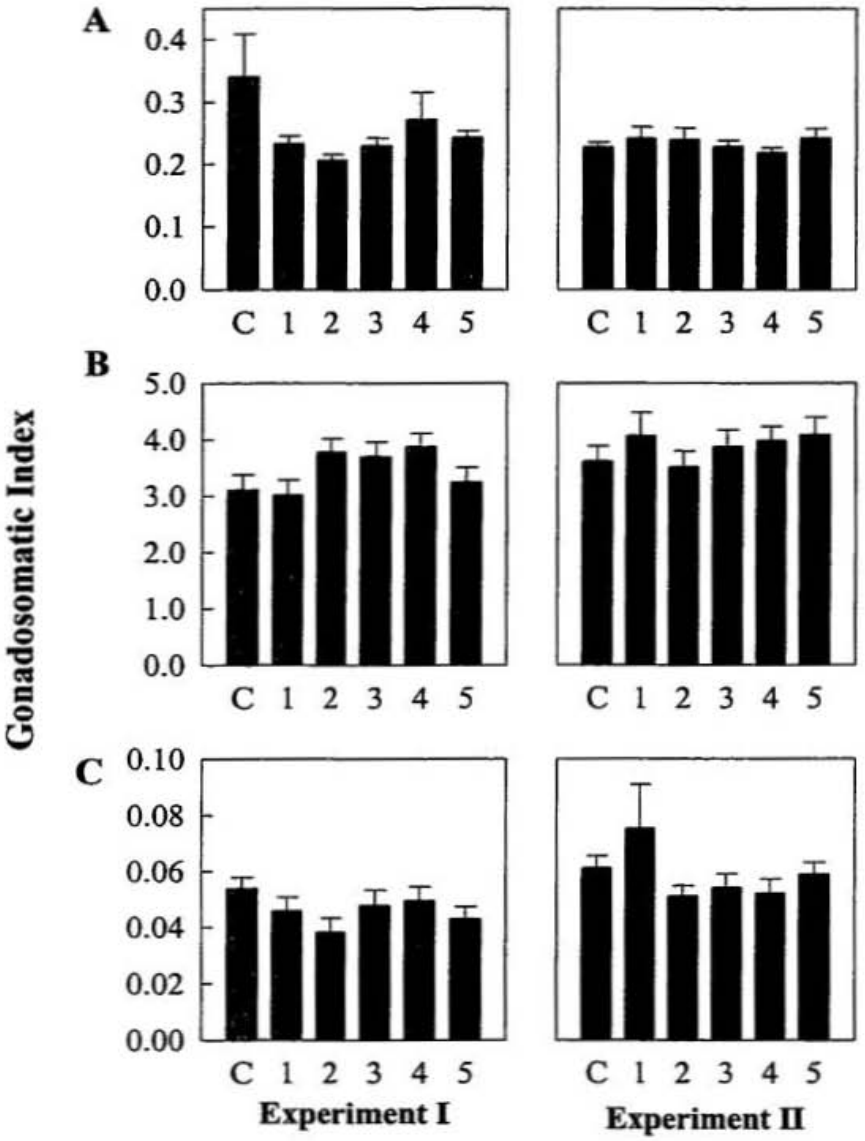


Figure 16: Mean gonadosomatic indices (July) of A) females, B) maturing males, and C) immature males in each group for Experiments I and II.

gonadal tissue investment or that subsequent feeding permitted catch up in losses of gonadal growth that may have occurred during starvation.

3.5 Effects of Food Restriction on Growth

3.5.1 *Final Fish Sizes*

Examination of the final size attained by all fish was conducted using three-way ANOVA to determine the contributions of replicate, treatment and sexual status on growth. Fish in all replicate treatments grew by a similar amount and attained similar mean sizes so data were pooled for evaluation of treatment and sex effects in further analyses (all replicate tank p-values > 0.05) (Appendix Tables A-3, A-4).

All size parameters differed significantly between treatment groups in both experiments and for sex in Experiment I (all p-values < 0.001) (Figures 17, 18). In Experiment II, only condition factor was significantly different between sexes ($p < 0.001$). Since there was obviously some influence of sex, fish were segregated by sexual status to examine the effects of treatment on growth.

Experiment I

Females of Group 5 were on average smaller that those of other groups, however, the observed differences were not statistically significant between treatments (weight: $F_{[5,374]} =$

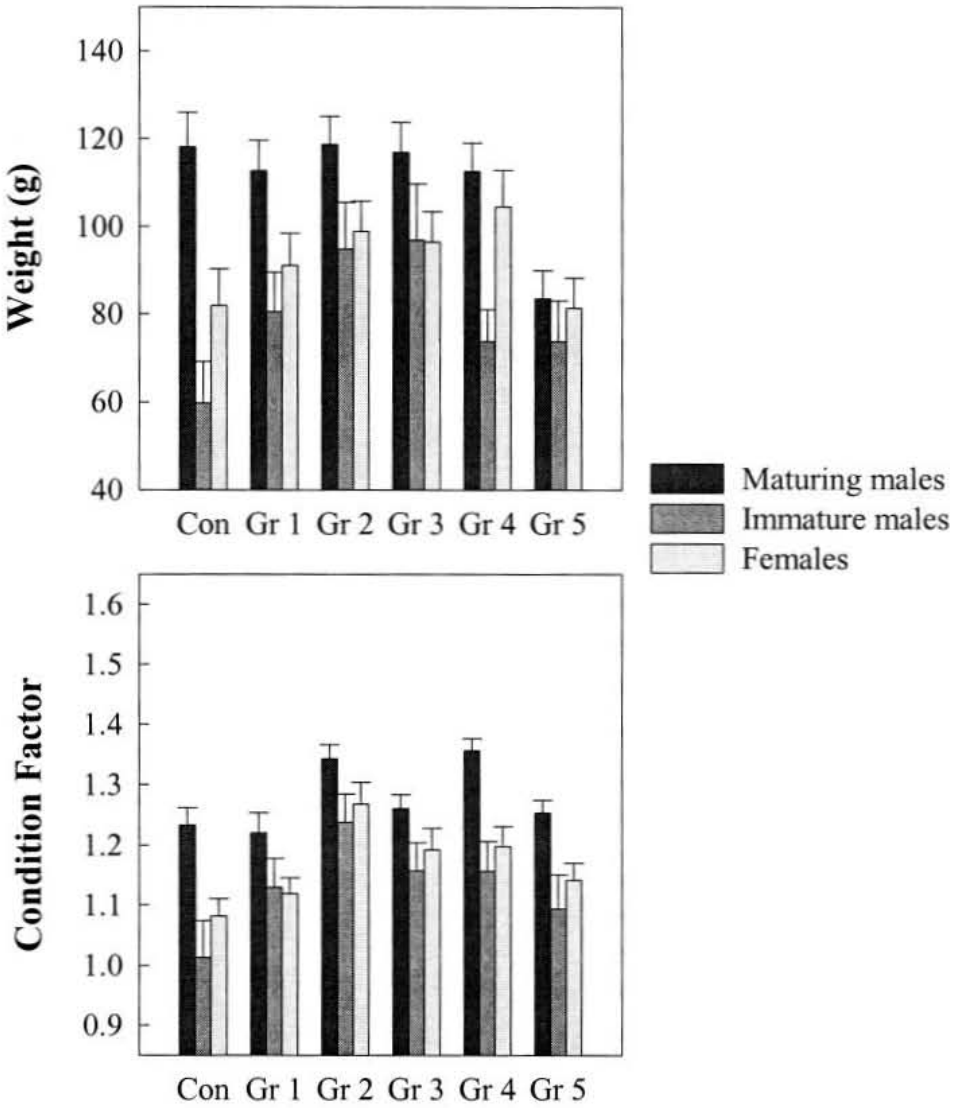


Figure 17: Mean (± s.e.) final weights and condition factors of all fish in Experiment I.

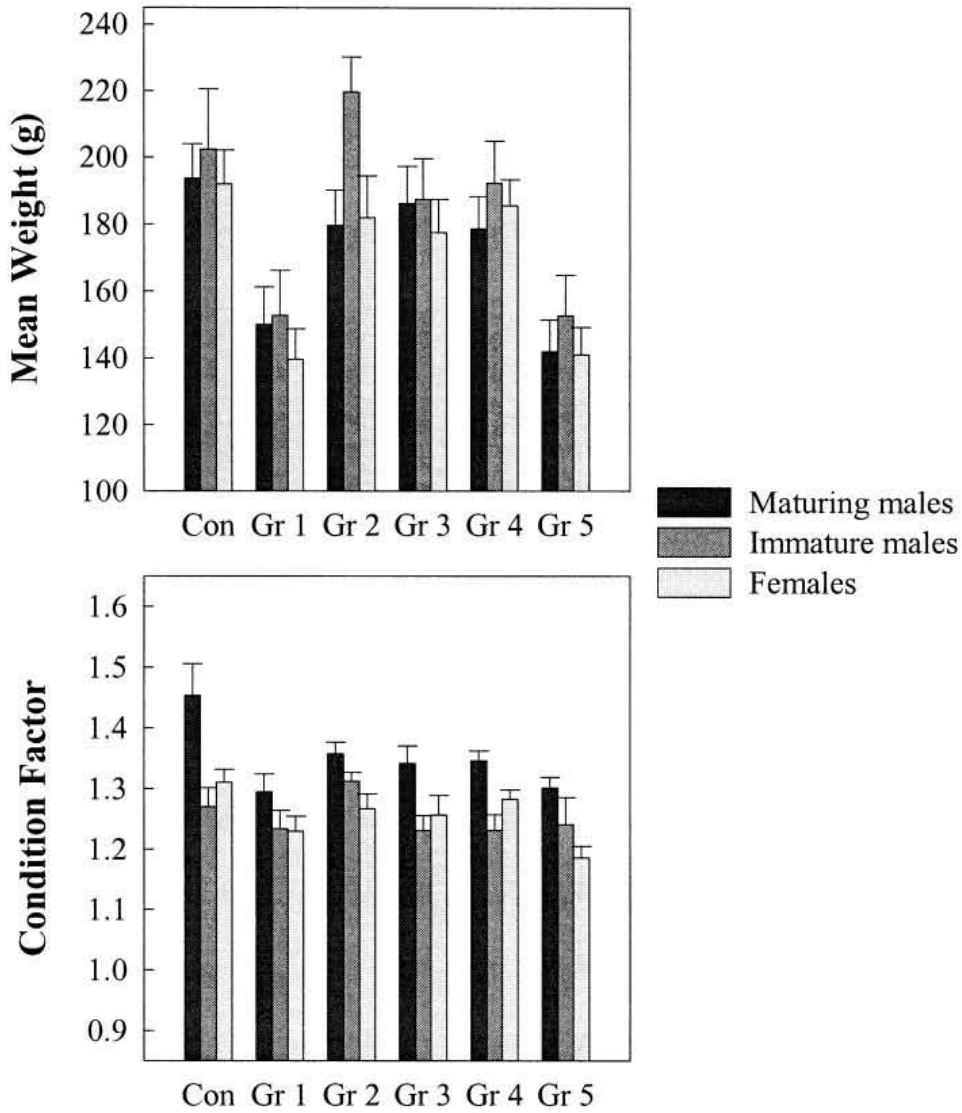


Figure 18: Mean (± s.e.) final weights and condition factors of all fish in Experiment II.

1.60, p = 0.158 ; length: $F_{[5,374]}$ = 1.52, p = 0.184). Treatment did, however, have a significant effect on condition factor ($F_{[5,374]}$ = 4.36, p = 0.001), with differences attributable to higher conditions associated with Group 2 fish (Tukey's). Overall, none of the restricted feeding regimes severely reduced growth in females.

Maturing males differed between groups for all size parameters (weight: $F_{[5,349]}$ = 2.89, p = 0.014; length: $F_{[5,349]}$ = 3.71, p = 0.003; CF: $F_{[5,349]}$ = 5.22, p < 0.001). Tukey's pairwise comparisons indicated that differences were attributable to Group 5, with final size in this group reduced in comparison to the other treatment groups. Immature males showed the same response to treatment (weight: $F_{[5,153]}$ = 2.32, p = 0.046; length: $F_{[5,153]}$ = 2.34, p = 0.044; CF: $F_{[5,153]}$ = 2.01, p = 0.080). Growth differences between treatments for immature males, however, could not be attributed to any specific group (Tukey's). It appears then, that males, especially when maturing, are more susceptible to losses of growth during times of food restriction than non-maturing females. Restricted feeding of maturing males during late spring seems to cause the most growth reduction, which is not surprising since it is at the time when energy reserves would be lowest after winter and there are higher metabolic demands occurring at this time due to increasing water temperatures and gonadal activity.

Experiment II

The restricted feeding regime used in Experiment II had significant effects on the growth of all fish. Treatment effects were observable in terms of weight, length and

condition for females (weight: $F_{[5,407]} = 6.01$, $p = 0.000$; length: $F_{[5,407]} = 4.99$, $p = 0.000$, CF: $F_{[5,407]} = 3.39$, $p = 0.005$), maturing males (weight: $F_{[5,286]} = 3.78$, $p = 0.002$; length: $F_{[5,286]} = 3.01$, $p = 0.011$, CF: $F_{[5,286]} = 3.51$, $p = 0.004$) and immature males (weight: $F_{[5,303]} = 4.21$, $p = 0.001$; length: $F_{[5,303]} = 4.87$, $p < 0.001$, CF: $F_{[5,303]} = 1.49$, $p = 0.192$). Again, most growth loss occurred in those groups which were subjected to starvation at higher temperatures (Groups 1 and 5).

3.5.2 *Individually Tagged Fish*

Since no females showed signs of maturation, analysis of the tagged fish was restricted to comparisons of growth differences between maturing and immature male fish of the control group and between groups for maturing and immature male fish.

3.5.2.1 Effect of Maturation on Growth

Experiment I

Growth patterns were different for maturing and immature fish in Experiment I. Analysis of Covariance (for ANCOVA results see Appendix Tables A-6 to A-9) of log transformed growth parameters regressed on time, showed significant interaction terms which indicate that the magnitude of the differences between maturing and immature males differed with time for weight (Fig.19A; $F_{(1,110)} = 24.31$, $p < 0.001$), length (Fig.19C; $F_{(1,110)} = 23.25$, $p < 0.001$) and condition factor (Fig.19B; $F_{(1,110)} = 11.87$, $p < 0.001$). Specific

growth rates of maturing fish were consistently higher than those of immature fish, this being indicated by the non-significant interaction of sexual status and time (Fig.19D; $F_{(1, 95)} = 0.32$, $p = 0.572$). According to this overall model, differences in mean SGR between the two groups were not significantly different ($F_{(1, 95)} = 2.79$, $p = 0.098$) even though the SGRs of maturing males were greater than those of immature fish. As it was of physiological interest to identify when growth patterns between maturing and immature fish diverged, one-way ANOVAs were conducted separately on measurements for each sampling time. This analysis indicated that maturing males were significantly larger in terms of weight by March ($F_{(1,17)} = 5.77$, $p = 0.028$) and in length by May ($F_{(1,17)} = 7.26$, $p = 0.15$), with differences widening as time progressed (Figure 19, A and C). At termination of this experiment in July, the average weight of the tagged maturing males was 151.37 ± 20.8 g and of the immature males 47.4 ± 13.3 g and the mean lengths were 22.0 ± 1.1 cm and 16.0 ± 1.0 cm, respectively.

Condition indices of maturing males were higher than those of immature males from the beginning of the experiment in November (Figure 19B), with significant differences detectable as early as January ($F_{(1,17)} = 18.09$, $p = 0.001$). From January to July, the condition of maturing males continued to increase, remaining significantly higher than the immatures. No increase in condition of immature fish was observed until July. Maturing fish grew at significantly higher rates than immature fish at all times with the exception of the period from June to July (Figure 19D). At this time, a faster increase in the SGR of immature fish

relative to the maturing fish occurred, decreasing the difference between the groups such that SGR values between them were no longer significantly different ($F_{(1,17)} = 0.06$, $p = 0.814$). This suggests that immature fish make up for sustained low levels of winter growth by rapidly increasing growth rates in summer.

Experiment II

Growth patterns observed in Experiment II were quite different from those of the previous experiment. Differences between maturing and immature fish were not so obvious as the magnitude of the differences between the groups over time did not change for any of the growth parameters (weight: Fig. 20A; $F_{(1,156)} = 2.04$, $p = 0.156$; length: Fig. 20C; $F_{(1,156)} = 1.08$, $p = 0.300$; condition factor: Fig. 20B; $F_{(1,156)} = 3.36$, $p = 0.069$; and specific growth rate: Fig. 20D; $F_{(1,136)} = 0.03$, $p = 0.866$). Maturing and immature male fish grew along the same slopes and had similar mean values at each sampling time. Despite there being no significant effects detectable in the overall model, condition indices of maturing males were observed to be greater than those of immature fish at each sampling time. However, variance of the data, especially among immature fish, was large so differences were only statistically significant in July (Fig. 20D: $F_{(1, 18)} = 4.87$, $p = 0.0434$).

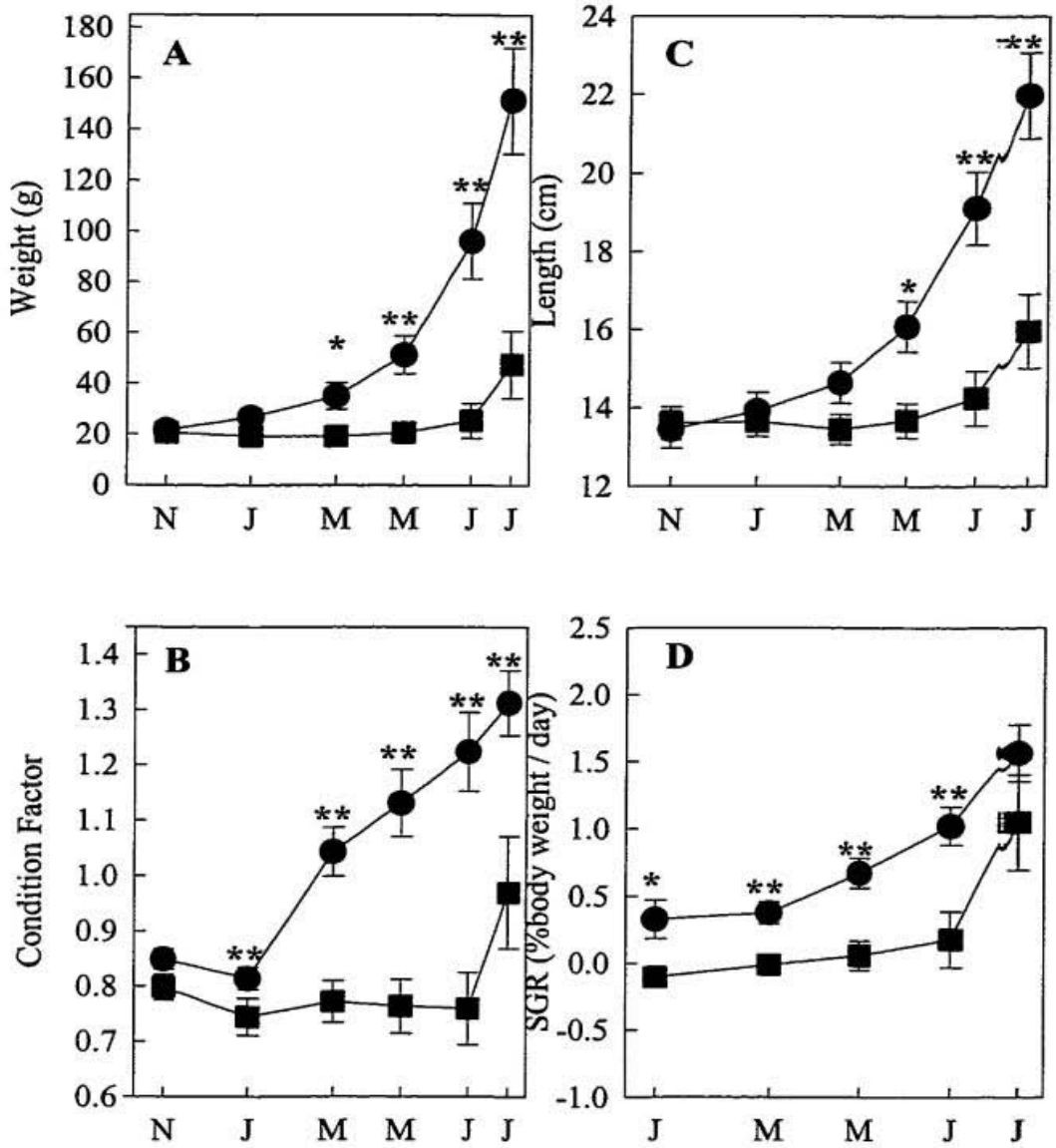


Figure 19: Comparison of mean growth parameters (± s.e.) over time between maturing (●, N = 11) and immature (■, N = 8) tagged males of Experiment I. Mean values below asterisks are significantly different from each other: * $p < 0.05$; ** $p < 0.01$.

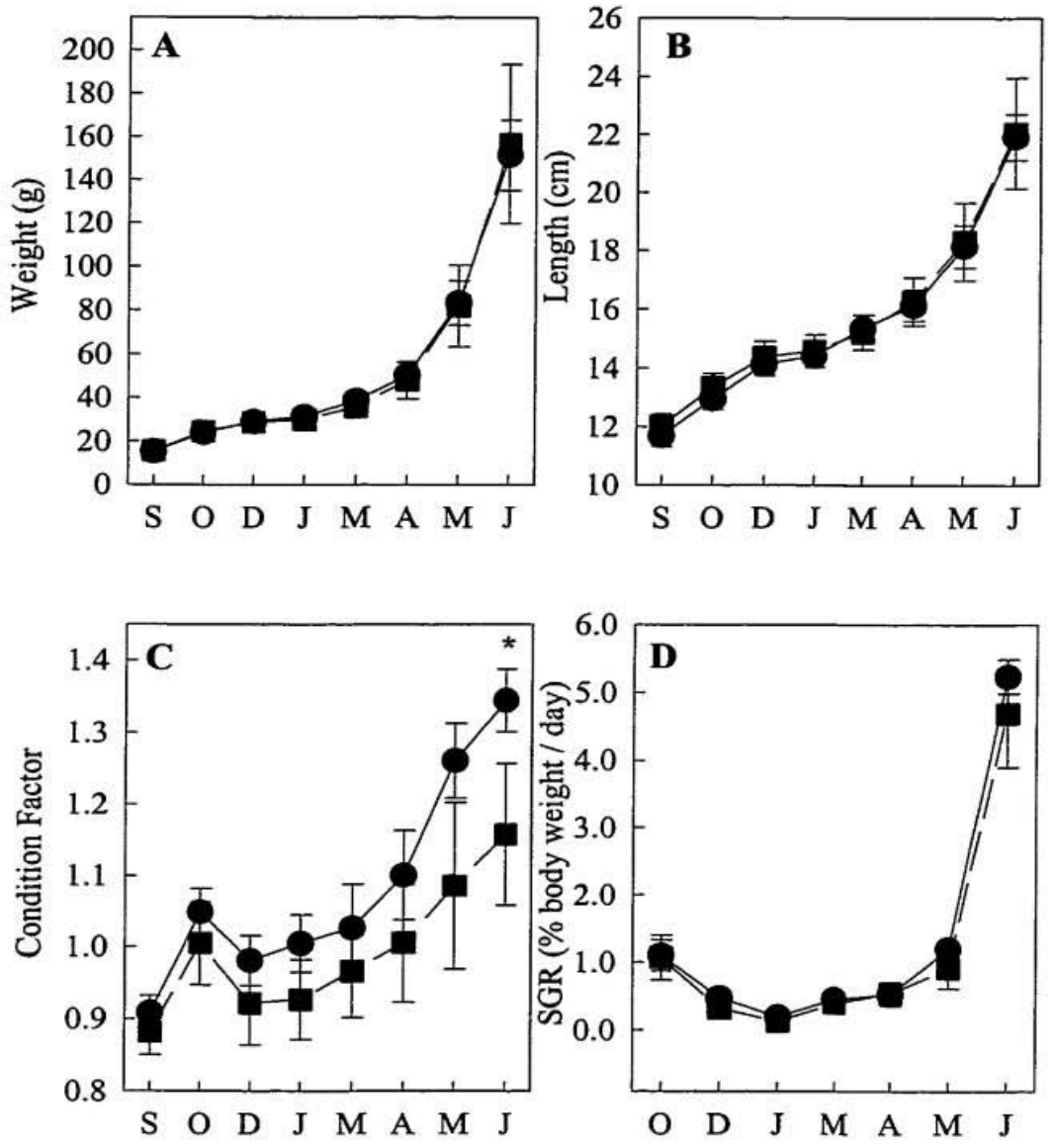


Figure 20: Comparison of mean growth parameters (± s.e.) over time between maturing (●, N = 11) and immature (■, N = 8) tagged male fish of Experiment II. Mean values below asterisks are significantly different from each other: * $p < 0.05$; ** $p < 0.01$.

3.5.2.2 Effect of Restricted Feeding Regime on Growth

Tagged individuals were used to follow growth responses of the fish to the restricted feeding regimes. Figures 21 to 23 illustrate these growth responses. Plots of length are not included but follow the same trend as those for weight. Growth responses were different for maturing and immature males and thus were analyzed separately.

Experiment I

In Experiment I, maturing fish of the control group were slightly larger at the termination of the experiment than all of the restricted fish. This difference was first observed in March and continued to increase thereafter. Analysis of Covariance (for ANCOVA results see Appendix Tables A-10 to A-13) of weight regressed on time showed that the magnitude of this difference varied significantly with time (Fig.21A; $F_{(5,372)} = 2.37$ $p = 0.039$). In July, significant differences in size between groups were detected and attributable to Group 5 differing from the control and from Group 3 ($F_{(5,63)} = 3.11$, $p = 0.015$). However, Group 5 was significantly different from the control at all times with the exception of the initial measurements taken in November. As this significance was observable before this group entered into its food restriction period, reduced feeding cannot be the only factor responsible for the smaller size of these fish.

Condition factor of maturing fish was affected by restricted feeding. The magnitude of differences between groups did not vary significantly over time (Fig.22A; $F_{(5,372)} = 0.52$,

$p = 0.758$), however, condition factor of maturing males of the control group had increased well above the other groups by March. Feed restricted groups maintained lower condition factors until June when an increase occurred in all groups, except the most recently starved Group 5. By July there were no significant differences in condition between any of the groups (Fig.22A; $F_{(5,58)} = 0.77$, $p = 0.575$). Specific growth rates of food restricted fish were low in all groups until temperatures began to increase in spring. Growth rates of the control group were slightly elevated over the other groups during the winter months but as temperatures increased, SGRs of fish fed on restricted regimes surpassed those of the control group (Fig.23A; $F_{(5,58)} = 4.67$, $p = 0.001$). Overall, it does not appear that restricted winter or spring feeding on a 2:2 regime results in any detrimental growth penalties on maturing fish. The fish seem to display a compensatory growth response during feeding subsequent to restricted periods, which is most pronounced as temperatures increase.

Analysis of Covariance of growth of immature fish indicated that weight did vary with time amongst groups (Fig.21B; $F_{(4,140)} = 8.57$, $p < 0.001$). Several groups of fish (Groups 1, 2, and 4) displayed slightly, but not significantly, greater final weight than the control and the other groups ($F_{(4,20)} = 2.29$, $p = 0.096$, one-way ANOVA). Condition factors varied greatly over time amongst immature fish with reductions in condition factor occurring in association with restricted feeding periods (Fig. 22B; $F_{(4,140)} = 4.07$, $p = 0.004$). However, the SGRs of immature fish did not show significant changes between groups over time (Fig. 23B; $F_{(4,115)} = 1.01$, $p = 0.403$), nor were elevations significant ($F_{(4,115)} = 0.18$, $p = 0.947$).

Thus, restricted feeding did not seem to significantly affect growth of immature fish. This is perhaps a result of immature fish having a lower demand for feeding in winter months than maturing fish. At termination, size and condition of immature fish were lower than maturing individuals, however, growth rates were comparable suggesting that they may be able to catch up for lost growth which occurred over the winter months.

In this experiment it was difficult to associate decreases in growth parameters directly with the restricted feeding periods because sampling of the tagged fish did not correspond directly with the starvation periods and also because of the overlapping of the restricted feeding periods of the various groups. In Experiment II, sampling was conducted immediately subsequent to each starvation episode and there was no overlapping of starvation times.

Experiment II

Maturing males from Experiment II displayed no significant size differences between groups over time (Fig. 21C; $F_{(5,548)} = 0.92$, $p = 0.471$) or in mean size attained at each sampling time ($F_{(5,548)} = 0.42$, $p = 0.835$). One-way ANOVAs conducted separately for each sampling time showed some significant differences occurring between various groups at different times with these being associated most often with groups that had been starved in the previous periods as would be expected. Condition factor showed a similar response to treatment as did size. Changes over time and at each time were not overall significantly

different between groups (Fig. 22C; $F_{(5,548)} = 1.53$, $p = 0.180$; $F_{(5,548)} = 0.42$, $p = 0.832$, respectively). However, it was observed that six week starvation periods were capable of reducing the condition factors of restricted fish in comparison with fed groups (Figure 22C). Subsequent feeding, however, allowed these reductions to be compensated for. The greatest reductions in condition in response to restricted feeding occurred in Groups 1 and 5, when starvation was imposed at times of higher temperature. The magnitude of the specific growth rates differed over time between groups (Fig. 23C; $F_{(5,478)} = 2.99$, $p = 0.011$). Restricted feeding drops the growth rate below the fed groups (Figure 23C); at each sampling time it is always the starved group which exhibits the lowest growth rate. This is not surprising as in this experiment restricted feeding consisted of complete starvation for six weeks with no intermittent feeding. In May, and at termination in July, SGRs of the different groups were still variable in magnitude indicating that the winter and early spring restricted feeding regimes affected subsequent growth rates of the groups differently. The July growth rates, however, are unusual in that there is a distinct segregation between the control and Groups 2 and 3, and between the other groups (Figure 23C). A similar pattern is seen for the immature fish (Figure 23D) suggesting that some other factor may be influencing growth at this time. The reduced growth rates in these tanks may be attributable to an early onset of PKD associated with increasing temperatures.

As shown in the comparison of growth between maturing and immature fish from Experiment II, growth of the immature fish from all groups was similar to their maturing

counterparts. Growth in weight was not significantly different between groups over time (Fig. 21D; $F_{(5,516)} = 0.96$, $p = 0.445$). However, mean weight of the immature fish did vary between groups ($F_{(5,516)} = 4.01$, $p < 0.001$). At final sampling, one-way ANOVA and Tukey's pairwise comparisons showed that significant weight differences amongst groups were small and were primarily attributable to differences between Groups 1 and 3 ($F_{(5,60)} = 2.57$, $p = 0.036$). Thus, it appears that regardless of differences in growth which occurred during restricted feeding, all immature fish were approaching similar size at the end of the experiment.

Changes in condition factor over time among immature fish was also insignificant (Fig 22D; $F_{(5,516)} = 0.43$, $p = 0.827$). This model also indicated that the mean condition factors amongst groups did not differ ($F_{(5,516)} = 2.13$, $p = 0.060$). However, again as with the maturing fish in this experiment, temporary reductions in condition were observable in those groups starved immediately prior to the measurement time.

Specific growth rates of immatures were also similar to maturing males in this experiment. The magnitude of differences between groups was significant indicating that the experimental groups grew at different rates over time (Fig. 23D; $F_{(5,450)} = 7.27$, $p < 0.001$). One-way ANOVA with Tukey's pairwise comparisons indicated that differences at individual sampling times between groups were largely attributable to reduced growth rates in the most recently starved groups. At post-starvation sampling times, May and July,

significant differences between groups were still detectable (May: $F_{(5,60)} = 17.17$, $p < 0.001$; July: $F_{(5,60)} = 17.64$, $p < 0.001$).

Although growth parameters between groups were not dramatically different in this experiment, nor were the final sizes attained, the six week starvation regime did prove to have at least a temporary effect on the performance of the fish in terms of condition and growth rates.

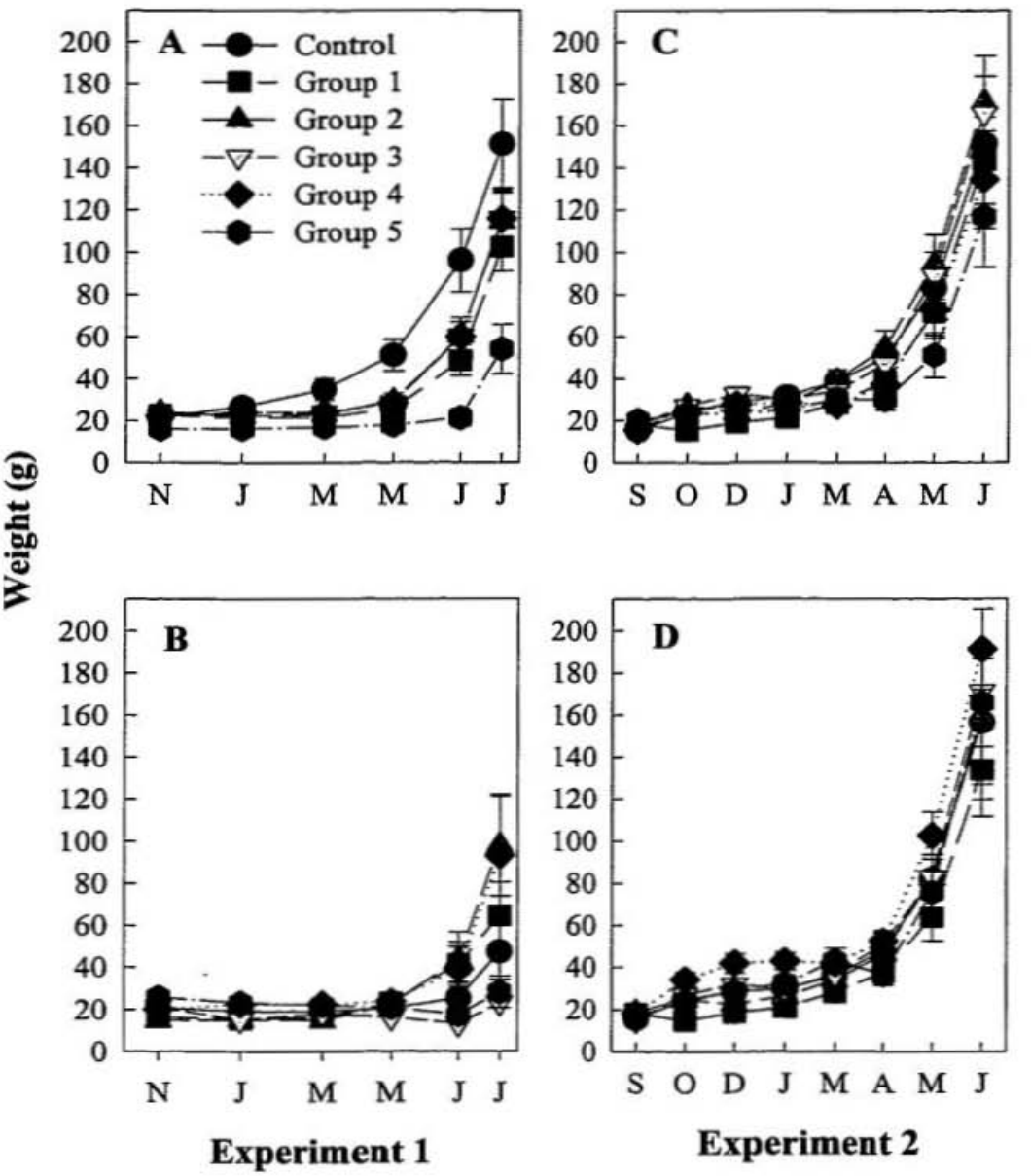


Figure 21: Mean weights (± s.e.) of maturing (A,C) and immature (B,D) tagged male fish from Experiments I and II. Symbols for all plots as defined for A.

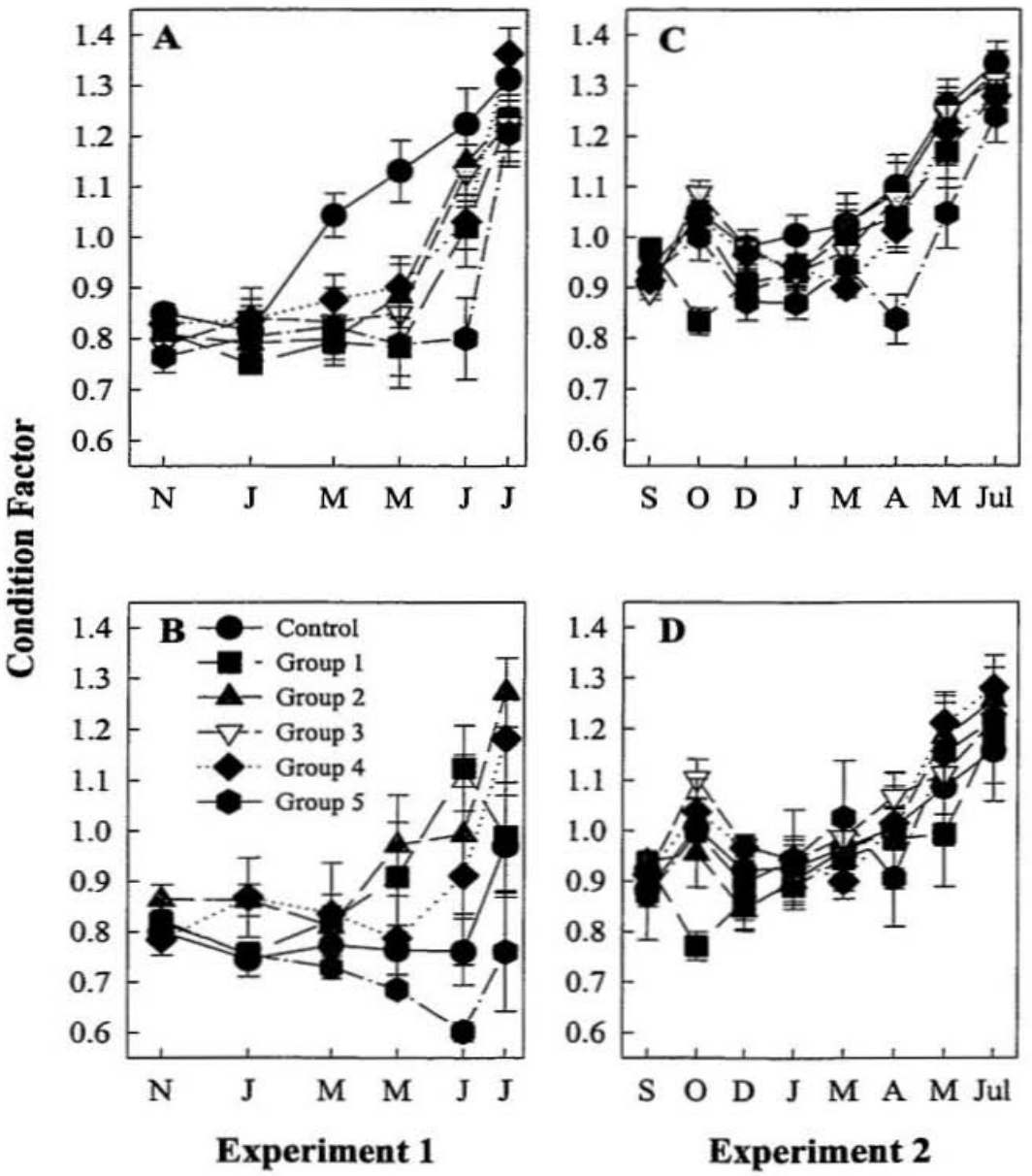


Figure 22: Mean condition factors (± s.e.) of maturing (A,C) and immature (B,D) tagged male fish from Experiments I and II. Symbols for all plots as defined for B.

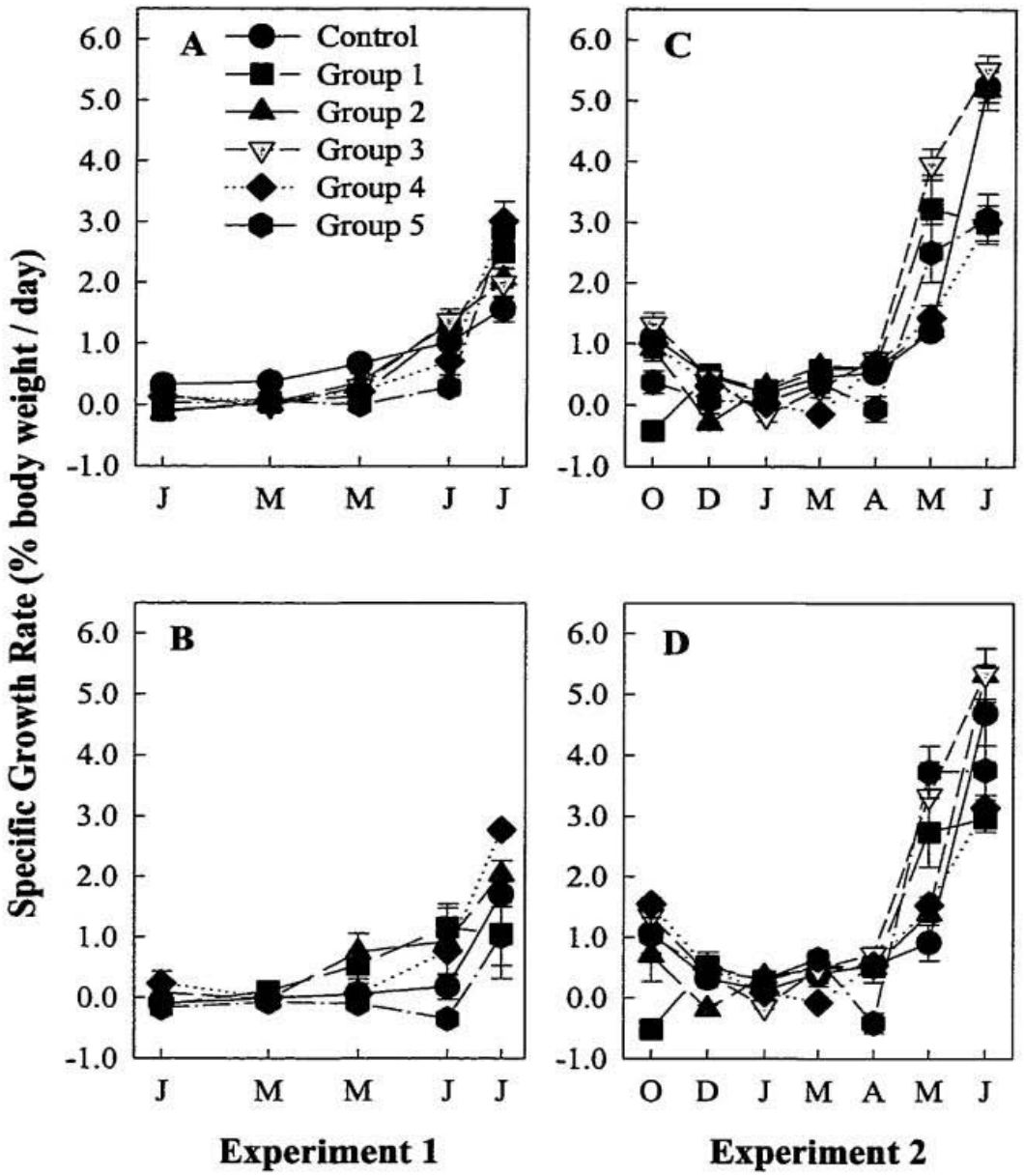


Figure 23: Mean specific growth rates (± s.e.) of maturing (A,C) and immature (B,D) tagged male fish from Experiments I and II. Symbols for all plots as defined for A.

4.0 DISCUSSION

4.1 SPERMATOGENESIS AND MATURATION

4.1.1 *Spermatogenic Cycle of Arctic Charr*

The microscopic anatomy of the testis and the process of spermatogenesis in Arctic charr is similar to that which has been described for other salmonids (Weisel 1943; Henderson 1962; Hiroi and Yamamoto 1968, 1969; Billard 1983,1986,1992; van den Hurk *et al.* 1978). Most of these descriptions focus on the later stages of active spermatogenesis once cysts and spermatocytes form and maturation is evident. Little reference has been made to a stage similar to what is described herein as the pre-spermatogonial stage (Stage I), where the testis is composed only of connective type tissue and testicular somatic cells with no distinct lobular structure or spermatogonia. Billard (1992) does mention a filament-like gonad which is supposedly an undifferentiated testis, where germ cells remain undifferentiated and few in number, and he cites Upadhyay (1977) as reporting that the male gonad of rainbow trout remains undifferentiated until the initiation of the first spermatogenic cycle, one or two years later. This corresponds well with Arctic charr in this study. However, in histological studies on the sex differentiation in rainbow trout, Takashima *et al.* (1980) reported that by 168 days post-hatch, the lobular arrangement of the testes is distinct with germ cells located within the lobules. Prior to this, the gonads were provisionally called

'second category', and were reported to be made up only of stroma cells whose features corresponded to prior descriptions of follicle cells and Sertoli cells (Billard 1986). It is possible this stage of testicular development is more commonly found in Arctic charr. Given that charr grow at slower rates and have a later age at maturity than rainbow trout (Delabbio 1995) this stage of sexual differentiation might extend later into the lifecycle, accounting for the occurrence of testes containing only somatic cells at age 1+. Billard (1986) reported that between the undifferentiated stage and lobule formation, that is, at the onset of puberty, testis size and the number of somatic cells increase. This again corresponds well with the charr examined in this study.

Miura *et al.* (1997) reported a similar state of testis development in the Japanese eel, *Anguilla japonica*. These eels, classified as Type 3, had non-lobed and thready testes with GSI values significantly less than other immature fish. Microscopically, there were no germ cells and the testes contained only connective-type tissue. They suggest that the lack of germ cells in these testes may have resulted from high temperatures and high density culture conditions. It is unlikely, however, that such an explanation could account for the pre-spermatogonial state of testicular development observed in the Arctic charr since the proportion of fish displaying such a state of testicular development decreased significantly between the autumn sampling periods and the final samples for both experiments. The most plausible explanation, therefore, is that the pre-spermatogonial stage as described herein for

Arctic charr, is a true stage of testicular development, a stage which occurs earlier than the immature stages.

An alternative explanation is that the small size of the fish and testis at this time may have led to losses of the portion of the testis which contained the germ cells. This is possible if the idea that the germ cells migrate from a permanent, localized germinal epithelium as has been proposed by some researchers (van den Hurk *et al.* 1978; Grier 1981) is true. However, personal histological observations of what appears to be lobular structure emerging from pre-spermatogonial testes and the renewal of germ cells from primary spermatogonia found along the lobular walls in fish undergoing a repeated maturation cycle makes the former explanation more likely.

Much uncertainty exists in the literature surrounding the characterization of the somatic cells of the testis of teleosts, especially as to the identification of Sertoli cells, Leydig cells, lobule boundary cells and cyst cells. Loir *et al.* (1995) separate the testes into germinal and interstitial compartments, which are separated by a basement membrane, the lobule wall, with which lobule boundary cells are reported to be associated (Lofts and Marshall 1957; Henderson 1962). The germinal component of the testis is found within the lobule and consists of only two cell types, the germ cells and Sertoli cells. Outside of the lobules, the interstitial tissue contains the connective tissue elements, blood vessels and Leydig cells. Based on this separation and on histological descriptions (van den Hurk *et al.* 1978) the darkly staining, ovoid, somatic cells of the pre-spermatogonial testes of Arctic

charr were classified as Leydig cells (Figure 2). This classification is supported by similar cells being identified outside the lobules in fish classified as immature (Figures 3, 4, 5). An interesting observation is that as testicular development proceeds, the number of these Leydig cells in the interstitium between adjacent lobules decrease such that during the later stages of spermatogenesis, they can no longer be seen (Figures 6, 7, 8, 9, 10). As well, it is interesting that during gonadal recrudescence, these cells reappear (Figure 13). This cycle of the Leydig cell is perhaps why it was thought that these typical endocrine interstitial cells of vertebrates were not present in the closely related brook charr, *Salvelinus fontinalis* (Henderson 1962).

Once the testes become structurally organized, primary spermatogonia can be seen in the testicular lobules of Arctic charr during all stages of the spematogenic cycle. Primary spermatogonia remaining along the lobular walls of the testis have been reported to be the origin of the annual supply of germ cells in brook trout (Henderson 1962), in sockeye salmon, *Oncorhynchus nerka* (Weisel 1943), and in several other teleosts. Henderson (1962) reported that these cells can be seen dividing at all times of the year except during the spawning season. Weisel (1943) observed these cells dividing at the end of each breeding season, forming cysts filling the lobules. In Arctic charr, the restoration of testicular structure by spermatogonial division was obvious in those fish which were previously mature and were undergoing a repetitive maturation cycle. During those months when residual spermatozoa were being phagocytosed, division of primary spermatogonia along the lobular

walls resulted in the production of primary and secondary spermatogonia. This is suggestive that Arctic charr do not maintain either an extra-testicular source of germ cells as has been reported from a single perch specimen (*Perca flavescens*) (Turner 1919) or a separate germinal epithelium from which germ cells arise and migrate (Foley 1926; Lofts and Marshall 1957) but rather that a permanent source of germ cells is maintained within each testicular lobule through the primary spermatogonia. Primary spermatogonia could be seen undergoing mitotic division and proliferation in immature fish from September through until July, but this was most obvious in both experiments from January through to the onset of Stage IV, when spermatocyte production begins (March - June).

It was hoped in this study that by identifying the stage, or stages, of spermatogenesis that are affected by nutrition, that a critical point in the spermatogenic cycle could be identified. This approach would be more useful than identifying when a critical period in terms of time (months or season, for example) occurs because it would make the results applicable to other species that undergo gonadal development on schedules different from Arctic charr. However, the timing of events in the spermatogenic cycle in the charr proved to be highly variable. The progression of spermatogenesis is asynchronous within the testis beyond the level of the individual cyst. Within a lobule of the testis of a maturing fish, cysts of cells at every stage of development may be identified up until the time when the testis is nearing completion of spermatogenesis and the lobules are filled with spermatozoa. This is complicated by the fact that the timing of the onset of maturation and advancement through

identifiable spermatogenic stages is highly variable among fish in the population. This is not the case for all fish. For example, winter flounder proceed through all spermatogenic stages within a strict time period during which testicular development in individual fish, and among fish of a given population, is very much synchronous (Harmin *et al.* 1995; Moulton 1998).

The variability in sexual development in salmonids may be an evolutionary strategy to deal with life in variable and unpredictable environments. Progressive development of the gonad, or postponement of the onset of active spermatogenesis, might be a strategy employed by individual fish to cope with harsh winters in terms of temperatures or food availability, allowing them to either reduce the extent of investment in reproduction or to skip reproduction for a given season altogether. The existence of such a strategy, however, would make it difficult to use nutritional manipulation for controlling the onset of maturation as has been successfully employed with other species such as with female winter flounder (Burton 1994). Nutritional inhibition of maturation requires that fish be deprived of food at the specific time when they are making the physiological 'decision' to begin or continue gonadal development. In fish such as winter flounder where progression through the gametogenic stages occurs only once annually during a short and limited season, depriving the animals of food may be sufficient to 'switch off' reproductive processes with no opportunity to resume gonadal development until the following year. In Arctic charr, and perhaps other salmonids, where the window of opportunity for gonadal development extends

over a longer time period, such a strategy would not be very successful unless the timing of food restriction was long enough to cover the entire range of time when gonadal development may commence. Fish used in this study were observed to enter into active spermatogenesis from March through until June, a four month period extending over the time when temperatures normally rise in spring and fish increase considerably in size. Theoretically, it may be possible to inhibit maturation by starving the fish over this entire period, but it is likely that a severe growth penalty would ensue.

Maturation in salmonids is believed to be a cyclical process that begins at fertilization and is regulated through inhibition (Thorpe 1986, 1994; Thorpe *et al.* 1998). That is, maturation is not switched on, but rather is continually repressed until such time that a, yet undefined, inhibitor is removed, allowing sexual development to proceed. The finding that some fish seemed capable of promoting only scattered germ cells through spermatogenesis to produce spermatozoa while the majority of the testis remained both macroscopically and microscopically immature is very interesting in terms of this inhibition factor. This is further evidence that a control mechanism regulating entry of the germ cells into cyst and spermatocyte production exists, and that once spermatocyte production is initiated, germ cells are committed to spermatogenesis through to the production of spermatozoa. It seems that spermatozoa such as these were produced either from spermatogonia that escaped the inhibition, breaking free from the controlling mechanisms inhibiting maturation or were cells that were permitted to develop when the inhibiting factor was temporarily removed before

a decision to proceed with maturation was reversed. It is highly unlikely that such spermatozoa were residual from the previous spawning season as they were observed late in the year (September) and were from testes that were classified macroscopically as immature, having a translucent nature and low (< 0.10) GSI value. An extensive literature search did not reveal references to other fish exhibiting similar minor testicular development. However, Miura *et al.* (1997) in studies on the induction of spermatogenesis by human chorionic gonadotropin (HCG) injection in Japanese eel found that some fish had testes that contained spermatogonia along with a small amount of spermatozoa that could not be hand stripped. They suggested that in these fish the spermatogenic process in most cells was interrupted during spermatogonial proliferation some time before entering meiosis.

4.1.2 *Nutritional Inhibition of Maturation*

Histological observations made on fish in Experiment II suggest that even if low nutrition is not able to stop maturation entirely for a given year in this species, restricting food intake may have temporary effects on testicular development by affecting mitotic proliferation of spermatogonia. Testes that were observed immediately after starvation periods had relatively fewer cells undergoing mitotic division than their fed counterparts. This, perhaps, is indicative of a nutritional control factor acting at the level of germ cell division to stop, or at least slow or delay, maturation in times of nutritional stress. However, this is simply an indication of a possible control mechanism at work, and additional studies

would need to be conducted to identify its exact nature to understand how nutrition affects mitotis and as well to quantify its effect.

4.1.3 *Critical Points in the Spermatogenic Cycle*

It has been generally accepted that gonadal growth in salmonids is initiated in spring under conditions of increasing photoperiod (Scott and Sumpter 1983). Thorpe (1986) proposed that maturation will occur if, during this time, the rate of accumulation of surplus energy exceeds a genetically determined threshold. Studies involving food restriction in spring have been use to extensively test this model with Atlantic salmon, however, the results are inconclusive. Reducing food intake and growth in spring has been found to be effective at suppressing maturation only in small proportions of populations and the timing of when food restriction proves most effective is variable. Thorpe *et al.* (1990) reported a 33% reduction in the proportion of maturing males when fish were fed every other week during February and March. Rowe and Thorpe (1990b) found that April and May and May and June were the most effective times, with reductions in the proportions of mature male parr from 93% in controls to 86 and 83%, respectively. They suggested based on these observations and increases in condition factor that occurred in April in maturing fish, that April represented a 'critical window' during which the decision to mature was made in this species. However, Herbinger and Friars (1992) found a non-significant reduction in maturation proportions of 14 and 18% from controls at 71 and 81% when fish were starved

from January to March and then maintained on a restricted diet until May. Berglund (1995), by reducing feeding levels in groups of fish during May and June, was able to reduce maturation in male salmon from 60% to 48, 45 and 36%. In amago salmon, *Oncorhynchus masou ishikawae*, restricted feeding in May reduced the incidence of early maturation in females, but male maturation was unaffected (Silverstein and Shimma 1994). Restricted feeding in autumn, winter or spring on a 2:2 regime was not sufficient to reduce maturation proportions of male Arctic charr in this study, although slight reductions were observed in direct comparison to controls in all groups starved for six continuous weeks in the second experiment. Relative reductions in the proportions of fish maturing in the experimental groups in comparison to the control in this experiment ranged from 5.1 to 12.7%.

The above model, which proved to be unsuccessful at explaining the onset of maturation in salmonids, has since been modified with the realization that the annual cycle of gonad growth actually commences in autumn, before endocrine changes are detectable (Thorpe 1994). Based on this, an alternate model of maturation in salmonids has been proposed in which there is not one, but two critical periods (Mangel 1994; Thorpe *et al.* 1998). The first critical period is reported to occur in November, when the fish makes an assessment of resources and the decision to either begin investment in gonadal tissue or to postpone development. That is, the decision to enter puberty is made at this time. The second critical period then occurs in spring when, provided lipid stores remain sufficiently high over winter and replenishment of lost reserves and good growth can occur in April and

May, fish decide whether or not to maintain maturation and spawn in the upcoming season, or to halt maturation and delay reproduction for another year. The existence of two critical periods in the salmonid maturational cycle also helps to explain why this and other attempts at preventing maturation solely by nutritional manipulation in spring, have been met with so much inconsistency.

The idea that there are two critical periods in the spermatogenic cycle of salmonids is supported in the histological observations made on Arctic charr in this study. If, what the results and observations of this study suggest is true, that (1) the first critical point occurs in autumn, affecting advancement of fish from a pre-spermatogonial stage of development into the immature stage, and (2) that a second critical point occurs in spring affecting cyst formation and the movement of cysts of secondary spermatogonia into spermatocyte production, then starvation or restricted feeding for short periods in either of these seasons could only affect the proportion of those fish for which that time is a critical period. In those species where the spermatogenic cycle is compressed into a shorter time period such as in winter flounder, two critical points in the spermatogenic cycle may still exist and could be affected by a single starvation period of relatively short duration.

A circannual endocrine switch from reproductive dormancy to active gonadal development, which is triggered by the energetic status of the fish, is suspected to exist for female walleye (*Stizostedion vitreum*) (Henderson *et al.* 1996). These investigators found evidence to suggest that in the gametogenic cycle of these fish there were two separate times

when an endocrine switch could operate: firstly, after energy reserves in the form of visceral fat are replenished by August, and secondly, in October when it was observed that even immature females exhibited a transient development of ovarian tissue. Although this switch is reported for females, it lends further support to the theory of there being two critical points in the maturation cycles of teleosts where the potential for successful reproduction, based on energy reserves, is assessed.

4.1.4 *Maturation Proportions*

Incidence of maturity was not reduced by restricted feeding during Experiment I when fish were fed on a regime of two weeks of starvation followed by two weeks of excess feeding over a duration of 14 weeks. In fact, when proportions of fish maturing in the experimental groups were compared directly to the control group, it was found that two of the groups had significantly higher proportions of maturing males. Thus, this type of feeding regime is not effective at inhibiting maturation. The second experiment, in which restricted feeding involved complete starvation for six week intervals had a different result. Groups of fish starved in autumn and in late spring each showed significantly reduced proportions of maturing males when groups were compared directly to control groups. It is interesting that these two periods correspond with the two possible critical points discussed above, however, further investigation would be necessary to confirm that these reductions resulted directly from nutritional deprivation affecting these two control points. It is possible that this

result is merely a reflection of starvation at both of these times occurring at times of elevated temperatures. Conducting such experiments under controlled temperature regimes would eliminate this query.

Between experiments, the proportion of fish maturing in the control groups was higher in the first experiment (64%) than in the second (56%). Although the fish were the same age, originated from the same strain and were obtained from the same hatchery, they were fertilized in different hatcheries. This may have given them slightly different growth histories and parental origins which could have affected the proportions capable of maturing in each year. Thorpe *et al.* (1983) found that the genetic composition of parents does have an influence on the proportion of fish showing early maturation. Alternatively, temperatures were higher in the second experiment, especially in late winter, which may have favored growth over maturation. This is supported by the fish used in Experiment II having higher overall growth and growth rates, attained earlier in the year, than fish from Experiment I. Thorpe *et al.* (1990), however, reported the opposite of this. With comparable populations of fish, growth and incidence of maturity between experiments was higher in the year when temperatures, and thus growth conditions, were more favorable. Other factors contributing to the observed difference in maturation proportions between years could be that in each year the fish selected were a representative sample of the mid-sized fish of the particular cohort. Growth histories between the years may thus have differed and had an effect on the proportions of fish at the different stages of testicular development for a given size. Finally,

the start and end dates of the experiments were not comparable. Experiment I began in November, and ended in late July; Experiment II was set up in September and ended in early July. Although it seemed that all fish which were going to mature for the current spawning season had already initiated gonadal development by July. This was apparent in the distinct segregation of GSI values between maturing and immature fish at that time, and there being very few fish which still had GSI values remaining in the questionable range of 0.10 to 0.20 by this time. However, the difference in termination dates, especially, may have influenced the final maturation proportions between years if some fish lagged significantly in gonadal development behind the other fish. This point must also be taken into consideration when examining the proportions of fish classified as maturing in feed restricted groups. It is possible that feed restriction delayed the timing of gonadal development but the lack of true significant differences in reductions of maturation proportions of feed restricted groups makes this unlikely.

4.1.5 *Gonadal Tissue Investment*

In these studies there was no influence of restricted feeding on the extent of gonadal investment in any fish, either females, maturing or immature males. Adams and Huntingford (1997) found that growth rate in the months leading up to maturation was predictive of the eventual GSI values in females but not in males. As well, Vøllestad and L'Abée-Lund (1994) examined data from 44 wild Arctic charr populations and found that gonadosomatic

index was not correlated with previous growth history. Previous growth history, however, does appear to influence the degree of investment of reproductive tissue in non-reproductive Atlantic salmon (Adams and Thorpe 1989).

4.2 Growth Responses

4.2.1 *Variation Between Experiments*

Growth patterns varied dramatically between experiments. Explanations as to why these differences exist are similar to those proposed above for the maturation proportions, with genetic and temperature variables perhaps contributing most significantly. It is most likely that the higher winter temperatures and earlier spring temperature rise observed in Experiment II was responsible for the larger size of the fish and higher growth rates attained. Although growth rates of Arctic charr at low temperature are greater than those of other salmonids (Tabachek 1991; Delabbio 1995) growth rates are known to increase dramatically with temperature. Wandsvik and Jobling (1982) found that growth rates in Arctic charr of the Hammerfest strain (Norway) increased from 0.29% body weight day^{-1} at 2.9°C to 1.4% day^{-1} at 13.1°C and Brännäs and Wilkund (1992) reported growth rates for Lake Hornavan (Sweden) Arctic charr at 0.3 °C to be 0.32% day^{-1} and 0.97% day^{-1} at 10°C. Mean overall growth rates in the Fraser River (Labrador) charr used in this experiment ranged from 0.25% day^{-1} in January (2°C) in both experiments to 1.25% day^{-1} in late July (14°C) of Experiment I and to 5.0% in early July (10°C) of Experiment II. The low growth rate at high

temperatures of fish in Experiment I in late July may be attributable to decreased growth due to the fish being affected by PKD at the higher temperatures or to maturing fish having entered a period of summer anorexia (Sæther *et al.* 1996; Tveiten *et al.* 1996). The reason for the dramatically increased growth rate in July of Experiment II is unknown, but most likely is attributable to stock differences or to elevated feeding during this early time of rapid temperature increase.

4.2.2 *Sexual Status and Growth*

It has been shown by several investigators that maturational status has an effect on growth in Atlantic salmon. Maturing fish are typically larger and increase weight in spring and early summer at a greater rate than immature fish (Hunt *et al.* 1982; Rowe and Thorpe 1990a,b; McLay *et al.* 1992; Forseth *et al.* 1994; Kadri *et al.* 1996). In addition, there is evidence to suggest that maturing males are already larger and have higher fat contents that non-maturing fish as early as October or November of the year prior to spawning (Rowe *et al.* 1991; Berglund 1992; Simpson 1992; Kadri *et al.* 1996, 1997). Bohlin *et al.* (1994) found that condition factor in late autumn was higher in maturing male parr in brown trout, one full year prior to spawning.

Growth differences between maturing and immature male Arctic charr have also been reported such that maturing male fish are typically larger than their non-maturing counterparts with differences being primarily attributable to differences in growth in winter

and early spring (Sæther *et al.* 1996; Tveiten *et al.* 1996; Adams and Huntingford 1997; Jobling *et al.* 1998). In general, the results of Experiment I agree with these reports. At the end of the first experiment, maturing males were larger in weight and had higher condition factors than either non-maturing males or females (Figure 17). Retrospective examination of growth over time in the marked individuals indicated that maturing males of the control group were significantly larger than immatures from March through to termination of the experiment in July. Condition factor and specific growth rates of maturing fish were higher than those measured for immature fish at all sampling times from November to July, however, it is interesting to note that between June and July, the increase in specific growth rate was higher in immature fish than in the maturing (Figure 19). This may be indicative of maturing fish either having reached a maximum growth rate or again to their entering into a period of summer anorexia reported to occur in maturing male salmonids (Berglund *et al.* 1992; Kadri *et al.* 1996, Simpson *et al.* 1996). It is likely, based on the final growth rates, that had the experiment not been terminated, the immature fish would have caught up and perhaps surpassed the maturing fish in size over duration of the summer as Hunt *et al.* (1982) found to have occurred with Atlantic salmon.

Fish in experiment II did not show a comparable growth response to those in the first experiment. There was no effect of sex or maturational status on the final size of the fish in any of the groups. Condition factors, however, were slightly higher in maturing males in all groups, with the difference most pronounced in the control group (Figure 18). The

individually tagged fish revealed that condition factors in the maturing males were higher than immature fish from the beginning of the experiment the previous September, with differences becoming slightly more pronounced as time progressed (Figure 20). Growth and growth rates were similar in all fish. The different growth patterns observed in this second experiment, again are perhaps reflective of higher temperatures during the winter months allowing non-maturing fish to feed and grow at rates comparable to the maturing individuals. However, in other experiments, growth differences between maturing and immature fish were clearly identifiable even when the fish were kept at a constant temperature of 4°C. (Tveiten *et al.* 1996; Sæther *et al* 1996).

4.2.3 *Restricted Feeding Regimes and Compensatory Growth*

After a period of restricted feeding, Arctic charr in this study displayed hyperphagia and compensatory growth responses as evidenced by increased growth rates in starved fish during their subsequent re-feeding periods. This response made up for the growth losses which occurred during the periods of restricted feeding such that by the end of the experiments, size differences between groups of fish were not dramatically different. The only differences which were observable were between groups starved at times of elevated temperatures (Group 5 in Experiment I and Groups 1 and 5 in Experiment II). At these times, the starvation periods would have had a greater impact on growth because of the higher metabolic demands associated with increased temperatures. Salmon deprived of food

supplies for up to two months have been able to recover body weight because of this phenomenon of compensatory growth (Reimers *et al.* 1993; Bull and Metcalfe 1997; Nicieza and Metcalfe 1997) and it has also been previously demonstrated in Arctic charr (Miglavs and Jobling 1989; Jobling and Miglavs 1993; Jobling *et al.* 1993).

The ability of fish to recover from times of food shortage through compensatory growth is beneficial in that growth is not lost, however, it is also this ability which may be responsible for periodic starvation not being a very effective method of controlling maturation in salmonids. If reproduction can be switched off by malnutrition at certain critical times, but compensatory growth following the period of malnutrition quickly and dramatically improves the nutritional status of the fish, a reversal might occur. That is, a sudden and dramatic improvement in feeding resulting in high levels of food intake, rapid growth and improvement of condition, might signal to the physiological mechanisms controlling maturation that conditions have changed and gonadal maturation should resume. This is especially true if, as it seems for Arctic charr, that the critical window(s) for initiation of maturation are wide. It appears that such a response occurred herein in the first experiment where restricted feeding consisted of two week alternating periods of excess feeding with starvation. During weeks of excess feeding between starvation episodes fish were probably consuming adequate food to offset the effect of starvation, especially when starvation was conducted under low temperatures and metabolic demands were low. Higher maturation proportions seen in food restricted groups compared to control fish supports this

theory. It is possible that work conducted by Thorpe *et al.* (1990) and Rowe *et al.* (1991), where restricted feeding was imposed on Atlantic salmon by alternating starvation with satiation feeding every other week, was also influenced by compensatory growth.

Starvation for six continuous weeks in the second experiment did seem to have a slight, but not necessarily permanent, effect on maturation. Starved groups showed reduced levels of gonadal activity and development immediately after the starvation period when compared to fed groups, but by subsequent sampling times, these differences were no longer detectable. It is likely that the compensatory growth response, occurring upon resumption of feeding, allowed for the make-up of lost growth and energy reserves at such a rate that gonadal development was able to resume in many of the inhibited fish. Alternatively, increased food intake during the hyperphagic period of re-feeding may actually have promoted fish which were on a later schedule of gonadal development into maturation. This is because the spermatogenic stages which may be susceptible to food deprivation do seem to endure, if not at the individual level, but definitely within the population for longer than six weeks.

4.3 Aquaculture Implications

Jobling *et al.* (1998) cites early maturation particularly amongst males as being one of the major constraints to the profitable culture of Arctic charr. The slowing or cessation of growth which accompanies maturation results from the fish becoming anorexic as

spawning approaches (Sæther *et al.* 1996; Tveiten *et al.* 1996) as fish become reliant on energy reserves which are mobilized and incorporated into the developing gonads (Jørgensen *et al.* 1997). Thus, it has been necessary to farm only late-maturing strains or monosex, all female stocks (Jobling *et al.* 1998). The ability to offset maturation by an alternative model, such as by nutritional regulation is of great interest to the aquaculture industry, not only for Arctic charr, but for other species in which early maturation interrupts production schedules of farmers. Such a method may prove preferable to alternate methods of preventing maturation such as the necessity to raise all female stocks or it may be useful as additional insurance against early maturity.

The results of this study, however, clearly suggest that controlling maturation simply by food manipulation will be difficult in Arctic charr and other salmonids. The extended duration of the spermatogenic 'critical' points in salmonids, their apparent flexibility in choice of life history strategies, the phenomenon of compensatory growth, variability in the timing of critical periods between species, strain and individuals and even environmental conditions between years will make the method unpredictable and unreliable. In addition, to completely offset maturation in a population, it may be necessary to starve fish for such extended periods of time that a growth penalty will surely result. It is recommended that until a better understanding of exactly how nutrition influences maturation in fish is characterized, that is, until the physiological mechanism linking nutrition with reproduction

is unraveled, that nutrition alone not be relied on as a method of preventing early maturation in salmonids.

The growth results of this study, however, do indicate that use of restricted feeding to maximize the benefits of compensatory growth response exhibited by fish may be profitable in terms of growth and economic benefits for Arctic charr culture as has been suggested for other aquaculture species (cod, Pederson and Jobling 1989; rainbow trout, Quinton and Blake 1990). However, it is suggested that restricted feeding not be severely imposed at high temperatures as growth may be lost.

4.4 Future Research Directions and Recommendations

The indication that there may be two critical points in the spermatogenic cycle of Arctic charr fits well with the theory of salmonid life history variation proposed by Thorpe (1994) and Thorpe *et al.* (1998), however, further research is required for confirmation. Associating histological observations of testes development with periods that are suggested as critical nutritional assessment times will be vital to our understanding of maturational processes in salmonids. This will greatly enhance opportunities for more precise research into the physiological control mechanisms that govern reproductive processes in fish. Identification of points in the gametogenic cycle that may be susceptible to nutritional status may be of increased importance to finding out how a control factor is working and what the nature of that control is, rather than simply identifying the timing of when life history

decisions are made. For example, the indication that mitosis of spermatogonia, and hence, testicular development can be slowed by starvation is suggestive that the controlling factor may be acting at the level of cell division. Exactly how nutrition affects mitosis, however, will require more careful analysis.

It is recommended that before further research into identifying a critical period in salmonids is conducted or before attempts are made to use nutrition as a method of controlling maturation in fish that increased effort be put into understanding the natural progression of spermatogenesis and that the links between nutrition and maturation be identified. In mammals, there is accumulating evidence that the protein leptin acts as a signal of nutritional status to the reproductive system (Ahima *et al.* 1997; Chehab *et al.* 1997). It is likely that an analogous signal is present in fish which acts to link nutrition to reproduction. Henderson *et al.* (1996) suggested that such a physiological mechanism for assessing the availability of lipid energy is acting in walleye.

Further investigation should also focus on the observation that gonadal development of salmonids is initiated in autumn. This possibility was first suggested by Thorpe (1994), however, he offered little evidence except to say that GSI values begin to increase in November. The histological observations made herein offer more substantial support to this suggestion, however, a more detailed analysis of gonadal development and the timing of the onset of maturation should be conducted for confirmation.

It has been demonstrated in Atlantic salmon that lipid reserves or the accumulation of lipids are important in determining maturation (Rowe and Thorpe 1990a,b; Rowe *et al.* 1991; Simpson 1992; Thorpe 1994; Kadri *et al.* 1996). An effect of lipid levels and body condition on maturation was also demonstrated in Arctic charr by Adams and Huntingford (1997). In this study, although, it was found that mean condition factors of maturing males were greater than those of immature males, condition factor could not be used successfully to predict which fish would eventually mature. This is because on the individual level, much variation between condition factor and eventual success at maturing existed. Some fish with high condition factors remained immature and others with very low measurements of body condition were able to mature; that is, no consistent relationships could be identified. It is likely that the simple measure of body condition used in this study is not sensitive enough to pick up differences between groups of fish. It would be beneficial in future studies to either monitor lipid levels in fish directly, or to use a more sensitive but un-obtrusive measure of body condition such as the method developed by Simpson *et al.* (1992) for Atlantic salmon which has more recently been shown to be applicable to Arctic charr (Adams *et al.* 1995).

5.0 CONCLUSIONS

The following conclusions can be drawn from this study:

1) Spermatogenesis in Arctic charr is initiated in autumn under decreasing photoperiods and is characterized by mitotic proliferation of spermatogonia before changes in GSI are detectable. The cycle begins approximately one year prior to spawning and is highly asynchronous within the testis of a single fish and between individuals of a population.

2) There appear to be two critical points in the spermatogenic cycle that may be susceptible to nutritional control. The first occurs in autumn, affecting the advancement of the pre-spermatogonial stage into the immature stages, or the entry of the fish into a pubertal period. The second occurs in spring affecting movement of fish through the transient stage and into the maturational stages and spermatocyte production. The fall critical point seems to be of shorter duration, and since it occurs earlier in the maturational cycle it may have more potential for use in controlling maturation by nutrition.

3) Restricted feeding in alternate two week periods did not reduce proportions of male fish maturing with respect to control groups. Several groups of fish fed on this regime had

maturation proportions above the control levels suggesting that such a regime may actually promote maturation by allowing fish increased opportunities for compensatory growth.

4) Starvation periods extending over six continuous weeks were observed to affect maturation in Arctic charr. Gonad samples taken from fish immediately after starvation periods showed reduced activity in comparison to fed groups. That is, starvation was observed to have an obvious, although temporary, effect on mitotic proliferation of spermatogonia during the early stages of gonadal development. Final maturation proportions of food-restricted groups were all reduced with respect to control groups. Reductions were statistically significant in two of the groups when the proportions found maturing were compared directly to the control groups.

5) Restricted feeding had no significant influences on the degree of gonadal investment in Arctic charr in terms of GSI by July.

6) In Experiment I, maturing fish were larger, were in better condition and grew at faster rates that immature fish. In Experiment II growth was comparable between maturing and immature fish, however, condition of maturing males was higher than immatures. Growth factors, however, could not be used successfully to predict maturation success.

7) Arctic charr show considerable capacity for compensatory growth after a period of food restriction provided food is not restricted at elevated temperature.

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APPENDIX

Table A-1: Final maturation proportions of the replicate tanks of fish in Experiment I. Binomial comparisons of frequencies were used to test for differences between replicates. P-values determined using a Chi-square distribution with df = 1.

Group	Tank 1			Tank 2			Totals			Standard Binomial		
	Mature	Immature	Total	Mature	Immature	Total	Mature	Immature	Total	G - test	p -value	Significance $\alpha = 0.05$
Control	27	16	43	30	16	46	57	32	89	0.0568	0.8116	NS
1	28	11	39	31	16	47	59	27	86	0.3386	0.5606	NS
2	34	11	45	36	12	48	70	23	93	0.0039	0.9505	NS
3	34	8	42	33	12	45	67	20	87	0.7169	0.3972	NS
4	27	15	42	28	12	40	55	27	82	0.3034	0.5817	NS
5	21	17	38	25	12	37	46	29	75	1.2013	0.2731	NS

Table A-2: Final maturation proportions of the replicate tanks of fish in Experiment II. Binomial comparisons of frequencies were used to test for differences between replicates. P-values determined using a Chi-square distribution with df = 1.

Group	Tank 1			Tank 2			Totals			Standard Binomial		
	Mature	Immature	Total	Mature	Immature	Total	Mature	Immature	Total	G - test	p -value	Significance $\alpha = 0.05$
Control	25	21	46	26	19	45	51	40	91	0.1087	0.7417	NS
1	22	27	49	20	28	48	42	55	97	0.1032	0.7481	NS
2	32	22	54	25	33	58	57	55	112	2.9338	0.0867	NS
3	23	23	46	26	30	56	49	53	102	0.1291	0.7194	NS
4	25	26	51	22	23	45	47	49	96	0.1291	0.9899	NS
5	26	27	53	19	29	48	45	56	101	0.0002	0.3382	NS

Table A-3: Three-way ANOVA results for the final growth parameters of all fish from Experiment I by tank[a], group[b], and sex[c]; S = significant, NS = not significant.

Source of Variation		df	Weight			Length			Condition Factor		
			F	p	Sig.	F	p	Sig.	F	p	Sig.
Main Effects	Tank	1	1.51	0.220	NS	1.60	0.206	NS	0.05	0.830	NS
	Group	5	3.72	0.002	S	4.04	0.001	S	9.07	<0.001	S
	Sex	2	18.5	<0.001	S	16.43	<0.001	S	28.27	<0.001	S
2-Way Interaction	Tank - Group	5	0.95	0.448	NS	1.15	0.333	NS	1.46	0.202	NS
	Group - Sex	10	1.34	0.203	NS	1.64	0.090	NS	0.78	0.650	NS
	Tank - Sex	2	0.90	0.407	NS	0.47	0.623	NS	0.75	0.474	NS
3-Way Interaction	Tank - Group - Sex	10	0.78	0.643	NS	1.01	0.437	NS	0.26	0.989	NS
Residual		849									
Total		884									

[a] Refers to replicates within treatment groups (1,2)

[b] Refers to treatment groups (c, 1-5)

[c] Refers to sex: maturing males, immature males or female

Table A-4: Three-way ANOVA results for the final growth parameters of all fish from Experiment II by tank[a], group[b], and sex[c]; S = significant, NS = not significant.

Source of Variation		df	Weight			Length			Condition Factor		
			F	p	Sig.	F	p	Sig.	F	p	Sig.
Main Effects	Tank	1	2.69	0.101	NS	3.83	0.051	NS	0.07	0.794	NS
	Group	5	11.73	<0.001	S	10.43	<0.001	S	4.98	<0.001	S
	Sex	2	0.94	0.390	NS	1.45	0.234	NS	23.4	<0.001	S
2-Way Interaction	Tank - Group	5	2.50	0.029	S	2.69	0.020	S	1.17	0.321	NS
	Group - Sex	10	0.53	0.872	NS	0.77	0.660	NS	1.47	0.147	NS
	Tank - Sex	2	0.03	0.974	NS	0.03	0.969	NS	0.10	0.902	NS
3-Way	Tank - Group - Sex	10	1.44	0.156	NS	1.23	0.270	NS	1.27	0.245	NS
Residual		975									
Total		1010									

[a] Refers to replicates within treatment groups (1,2)

[b] Refers to treatment groups (c, 1-5)

[c] Refers to sex: maturing males, immature males or female

Table A-5: Final measurements of maturing and immature male fish in Experiment I (Table A) and Experiment II (Table B). Data shown are mean values ± standard error of the mean.

A

Group	Maturing males				Immature males			
	N	Weight (g)	Length (cm)	CF	N	Weight (g)	Length (cm)	CF
Control	57	118.1 ± 7.9	20.5 ± 0.5	1.23 ± 0.03	32	59.8 ± 9.5	16.7 ± 0.7	1.01 ± 0.06
1	59	112.2 ± 7.0	20.4 ± 0.4	1.22 ± 0.03	27	80.5 ± 9.0	18.4 ± 0.6	1.13 ± 0.05
2	70	118.8 ± 6.4	20.2 ± 0.4	1.34 ± 0.02	23	94.9 ± 10.6	18.9 ± 0.7	1.23 ± 0.04
3	67	116.9 ± 6.9	20.3 ± 0.4	1.26 ± 0.02	20	96.9 ± 12.8	19.3 ± 0.8	1.16 ± 0.05
4	55	112.6 ± 6.5	19.8 ± 0.4	1.36 ± 0.02	27	73.7 ± 7.3	17.8 ± 0.5	1.16 ± 0.05
5	46	83.5 ± 6.4	18.2 ± 0.5	1.25 ± 0.02	29	73.7 ± 9.3	17.8 ± 0.6	1.10 ± 0.06

B

Group	Maturing males				Immature males			
	N	Weight (g)	Length (cm)	CF	N	Weight (g)	Length (cm)	CF
Control	51	193.7 ± 10.3	23.4 ± 0.5	1.45 ± 0.05	40	202.4 ± 18.1	23.9 ± 0.8	1.27 ± 0.03
1	42	150.0 ± 11.3	21.7 ± 0.6	1.30 ± 0.03	55	152.8 ± 13.4	21.8 ± 0.7	1.23 ± 0.03
2	57	179.6 ± 10.5	22.9 ± 0.5	1.36 ± 0.02	55	219.7 ± 10.5	25.2 ± 0.4	1.31 ± 0.01
3	49	186.2 ± 11.1	23.5 ± 0.5	1.34 ± 0.03	53	187.5 ± 12.2	23.9 ± 0.5	1.23 ± 0.02
4	47	178.6 ± 9.6	23.2 ± 0.5	1.35 ± 0.02	49	192.3 ± 12.6	24.2 ± 0.6	1.23 ± 0.03
5	45	141.9 ± 9.5	21.6 ± 0.5	1.30 ± 0.02	56	152.7 ± 12.1	22.1 ± 0.6	1.24 ± 0.04

Table A-6: ANCOVA results for weight of individually tagged Arctic charr of sex[a] regressed on time.

Weight		Experiment I			Experiment II	
Source of Variation	df	F	p	df	F	p
Sex	1	0.01	0.905	1	0.04	0.848
Time	1	68.33	< 0.001	1	189.97	< 0.001
Sex - Time	1	24.31	<0.001	1	2.04	0.156
Residual	110			156		
Total	113			159		

[a] sex refers to maturing or immature males

Table A-7: ANCOVA results for length of individually tagged Arctic charr of sex[a] regressed on time.

Length		Experiment I			Experiment II	
Source of Variation	df	F	p	df	F	p
Sex	1	0.82	0.367	1	0.43	0.512
Time	1	66.06	< 0.001	1	228.56	< 0.001
Sex - Time	1	23.25	<0.001	1	1.08	0.300
Residual	110			156		
Total	113			159		

[a] sex refers to maturing or immature males

Table A-8: ANCOVA results for condition factor of individually tagged Arctic charr of sex[a] regressed on time.

Condition Factor	Experiment I			Experiment II		
Source of Variation	df	F	p	df	F	p
Sex	1	3.97	0.049	1	0.89	0.348
Time	1	36.94	< 0.001	1	37.63	< 0.001
Sex - Time	1	11.87	<0.001	1	3.36	0.069
Residual	110			156		
Total	113			159		

[a] sex refers to maturing or immature males

Table A-9: ANCOVA results for specific growth rate of individually tagged Arctic charr of sex[a] regressed on time.

Specific Growth Rate	Experiment I			Experiment II		
Source of Variation	df	F	p	df	F	p
Sex	1	2.79	0.098	1	0.08	0.784
Time	1	45.10	< 0.001	1	63.44	< 0.001
Sex - Time	1	0.32	0.572	1	0.03	0.866
Residual	95			136		
Total	98			139		

[a] sex refers to maturing or immature males

Table A-10: ANCOVA results for weight of individually tagged Arctic charr of the different treatment groups[a] regressed on time. Table A, Experiment I; Table B, Experiment II.

A

Weight - Experiment I	**Maturing males**			**Immature Males**		
Source of Variation	df	F	p	df	F	p
Group	5	0.88	0.498	4*	4.58	0.002
Time	1	332.49	0.000	1	50.62	< 0.001
Group - Time	5	2.37	0.039	4	8.57	< 0.001
Residual	372			140		
Total	383			149		

* Group 3 excluded because N = 1.

B

Weight - Experiment II	**Maturing males**			**Immature males**		
Source of Variation	df	F	p	df	F	p
Group	5	0.42	0.835	5	4.01	< 0.001
Time	1	293.68	<0.001	1	577.96	0.001
Group - Time	5	0.92	0.471	5	0.96	0.445
Residual	548			516		
Total	559			527		

[a] Refers to treatment groups (c, 1-5)

Table A-11: ANCOVA results for length of individually tagged Arctic charr of the different treatment groups[a] regressed on time. Table A, Experiment I; Table B, Experiment II.

A

Length - Experiment I	**Maturing males**			**Immature Males**		
Source of Variation	df	F	p	df	F	p
Group	5	1.11	0.353	4*	8.56	< 0.001
Time	1	269.47	< 0.001	1	65.99	< 0.001
Group - Time	5	3.21	0.007	4	8.21	< 0.001
Residual	372			140		
Total	383			149		

* Group 3 excluded because N = 1.

B

Length - Experiment II	**Maturing males**			**Immature males**		
Source of Variation	df	F	p	df	F	p
Group	5	0.76	0.577	5	3.82	< 0.001
Time	1	520.32	< 0.001	1	722.13	< 0.001
Group - Time	5	1.83	0.105	5	1.24	0.291
Residual	548			516		
Total	559			527		

[a] Refers to treatment groups (c, 1-5)

Table A-12: ANCOVA results for condition factors of individually tagged Arctic charr of the different treatment groups[a] regressed on time. Table A, Experiment I; Table B, Experiment II.

A

Condition - Experiment I	**Maturing males**			**Immature Males**		
Source of Variation	df	F	p	df	F	p
Group	5	2.06	0.069	4*	0.13	0.972
Time	1	209.93	< 0.001	1	15.99	< 0.001
Group - Time	5	0.52	0.758	4	4.07	0.004
Residual	372			140		
Total	383			149		

* Group 3 excluded because N = 1.

B

Condition - Experiment II	**Maturing males**			**Immature males**		
Source of Variation	df	F	p	df	F	p
Group	5	0.42	0.832	5	2.13	0.060
Time	1	202.36	< 0.001	1	125.66	< 0.001
Group - Time	5	1.53	0.180	5	0.43	0.827
Residual	548			516		
Total	559			527		

[a] Refers to treatment groups (c, 1-5)

Table A-13: ANCOVA results for specific growth rates of individually tagged Arctic charr of the different treatment groups[a] regressed on time[b]. Table A, Experiment I; Table B, Experiment II.

A

SGR - Experiment I	**Maturing males**			**Immature Males**		
Source of Variation	df	F	p	df	F	
Group	5	3.20	0.008	4*	0.18	0.947
Time	1	404.95	< 0.001	1	42.87	< 0.001
Group - Time	5	3.55	0.004	4	1.01	0.403
Residual	308			115		
Total	319			124		

* Group 3 excluded because N = 1.

B

SGR - Experiment II	**Maturing males**			**Immature males**		
Source of Variation	df	F	p	df	F	p
Group	5	0.86	0.510	5	2.45	0.033
Time	1	287.26	< 0.001	1	255.68	< 0.001
Group - Time	5	2.99	0.011	5	7.27	< 0.001
Residual	478			450		
Total	489			461		

[a] Refers to treatment groups (c, 1-5)